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EVOLUTIONARY CHANGES IN COMPONENTS OF FITNESS AND OTHER POLYGENIC TRAITS IN *DROSOPHILA MELANOGASTER* POPULATIONS

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1. INTRODUCTION

CHARLES DARWIN's theory and current genetic interpretations regard natural selection as the main determinant of evolutionary events. In the course of succeeding generations, a population copes with a given environment through a process of trial and error. The errors, that is, the variations which are less fitted to the environment, are lost, successful trials or variations better fitted are preserved; the process responsible for the fate of different variations is based on the fact that unlike types leave different average numbers of adult progeny in the next generation. If such unlikeness is at least partly hereditary the variations responsible for it persist for longer or shorter periods. In the course of time the population thus becomes better adapted to its surroundings, and this adaptation is made apparent by a series of changes in the morphology and physiology of the organism concerned. These changes in time are generally referred to as evolution.

Morphological and physiological characters which may undergo change fall under the control of genetic determinants which, for the sake of simplicity, have been classified by Mather (1943) under two main categories: unifactorial (or oligogenic) and multifactorial (or polygenic). Evolutionary change may thus be described either (1) in terms of variations in frequency of those genes that primarily affect single traits, or (2) in terms of variations in the mean values of morphological and physiological characters that show continuous variability and are controlled by polygenes.

While most of our genetic knowledge on evolutionary processes is derived from the study of variations in the frequency of oligogenes and chromosomal types, the significance of changes of the type indicated under (2) needs be stressed because of the following facts. In the first place, most of the paleontological evidence on the historical process of evolution is inferred from successions of fossils differing in shape and size, and the traits concerned were most likely controlled by polygenic systems. In the second place, zoo- and phyto-geographic data indicate that living organisms may differ, over their distribution

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range, in morphological and physiological traits which are considered to be adaptive and are known to be mostly of polygenic type. In the third place, the components of fitness, such as fecundity, fertility and the like, fall under the control of polygenes in the majority of the studied forms.

In spite of the importance of polygenic characters in evolution, however, no experiments have been reported thus far, which might show how natural selection operates on polygenic systems over a number of generations. The work reported here is an attempt to analyse the changes in such systems which natural selection can produce in experimental populations.

2. TECHNIQUES FOR BREEDING POPULATIONS

The classic paper of L'Héritier and Teissier (1934) was the first to demonstrate the effects of natural selection on single gene differences present in artificial populations of *Drosophila melanogaster*. Since that time variations in the frequencies of genes and of chromosome types, produced by natural selection, have been described in many *Drosophila* species kept in population cages. These are small boxes in which the amount of food available to the population is kept relatively constant; that is, every day or every few days fresh food is introduced and the worked-out food is removed. The population of the cage will reach an approximately stationary number, regardless of the original number of flies introduced, provided that the supply of fresh food as well as other environmental factors, such as temperature, remain constant. The amount of available food at any particular time determines the number of flies that reach the adult stage. This number is smaller than the number of zygotes represented by the fertilised eggs because of mortality in the larval and pupal stages. The struggle for existence and reproduction may also occur in the adult stage because of high population density. The genotypes better adapted to the prevailing conditions will accordingly become more frequent than those less well adapted.

Different experimental techniques have been used by other authors. In order to evaluate the relative selective values of eleven mutants of *D. melanogaster*, Wiedemann (1936) crossed them with wild-type flies. In the F_1, F_2 and succeeding generations the first 50 adults to emerge were used as parents of the following generation. A comparison between the observed frequencies of various phenotypes and the ones calculated for Mendelian ratios in succeeding generations, using Jennings' formulas, provided a measure of the relative viability of the various mutants. Nikoro and Gusev (1938), with a similar technique, kept populations of flies with various genotypes in large bottles and collected at random in each generation 100 pupæ, which were transferred to a new bottle where they could hatch and breed. Kalmus (1945) started an experiment with five homozygous wild-type females and five homozygous *ebony* males, in one breeding bottle. The progeny was counted and 150 flies were chosen at random and subdivided into 5 groups of thirty flies each to become parents of the following generation in five separate bottles. Records were kept of the numbers of wild-type and ebony flies appearing in each generation. This procedure was repeated for several generations in eight parallel series kept at different temperature and humidity conditions. Reed and Reed (1948) used the "population bottle," consisting of two half-pint milk bottles, the mouths of which were connected by rubber tubing. Each milk bottle had a paper cap perforated with two or three quarter-inch holes which allowed passage of the flies but prevented food and debris from being pushed from one bottle to the other by larvæ. The experiments were started with five pairs of flies in one

bottle, the second bottle being added after two months. Two months later the first bottle was removed and a fresh one substituted. By repeating this operation the population can be kept going at will. Techniques such as these seem to be less reliable for a thorough study of natural selection phenomena than the population cages of L'Héritier and Teissier because : (*a*) the population number is smaller and thereby random fluctuations in the frequency of a gene may give a biased picture of the selection process ; (*b*) a periodically changing environment (such as that of the " population bottle ") or arbitrary choice of parents in each generation may emphasise some and neglect other of the viability factors which are of significance for the competitive success of a given genotype.

The technique used in the work to be reported here is more laborious than those previously described, but provides a wider range of information on the dynamics

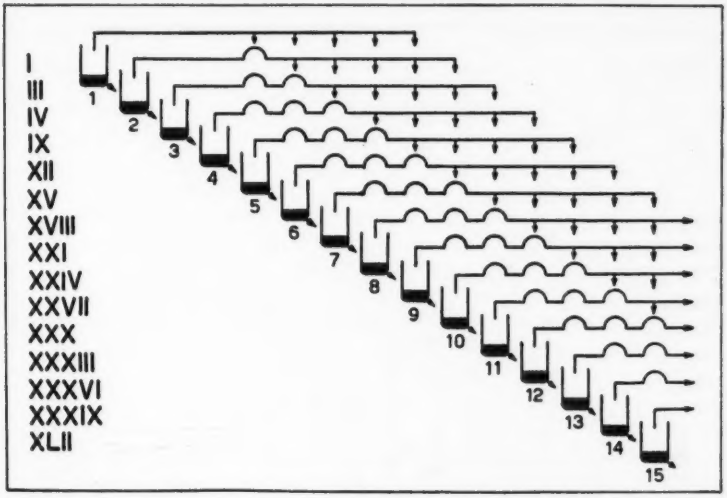

FIG. 1.—Schematic representation of the breeding technique used for the experimental populations. The vials are indicated with successive Arabic numbers ; the arrows protruding from them indicate the transfer of the adult individuals from one vial to the next, which occurred every three days (indicated in Roman numbers). The lines originating from the vials and the downward arrows indicate that the adult progeny of each vial was added to the population ; there are three arrows for each vial to show that the adults hatching from each vial were collected and added to the population over a period of 15 days (3×5) ; after that the vial was discarded.

of the populations under study. This method, already described in an earlier paper (Buzzati-Traverso, 1947*a*), takes advantage of the knowledge derived by Pearl (1927) while studying the growth of Drosophila populations. The experiment is started by introducing a certain number of adult flies of the kinds desired into a vial with food. After one, two or three days, according to the type of experiment one wishes to carry out, the flies are transferred to a new vial of the same size and with the same amount of fresh food. The transfer is repeated to new vials with the chosen constant periodicity. With a three day periodicity the number of flies in the first three vials of the series will be the same, unless some die in the meantime. At 25° C., adults of *Drosophila melanogaster* emerge on the ninth day after eggs have been laid, and therefore, some young flies will be found in the first vial on the tenth day from the beginning of the experiment. These are counted, and added to the original group of flies, which, by this time, will be in the fourth vial.

Every day the flies emerging in the earlier vials are transferred to the last one, which, as time passes, will have an increasing number of adult individuals (fig. 1). Such increase will continue up to a point when an equilibrium is reached between the amount of available food and space and the number of individuals which can live under such conditions. The equilibrium will be an approximately stationary one under constant conditions, although, as it will be shown later, this is not always true.

By means of this procedure, one can obtain : (1) data on the number, sex and phenotypic ratios of the flies present in the population at the time of each transfer to a new vial ; (2) data on the birth rates of the two sexes and of the phenotypes in each vial. This information makes it possible to evaluate at any particular time : (*a*) variations in the population size ; (*b*) variations in the sex ratio of the population as a whole or of its constituent phenotypes ; (*c*) variation in the gene frequencies ;

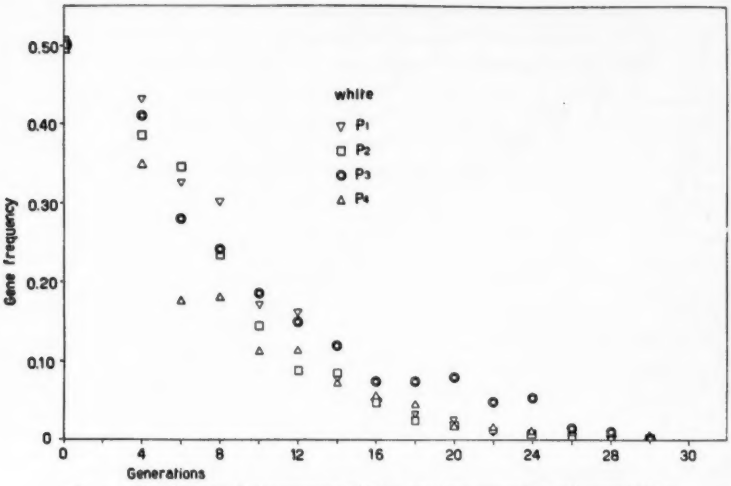

FIG. 2.—Elimination of the mutant *white* in the four experimental populations (P_1, P_2, P_3, P_4).

(*d*) the mean life length of adult individuals ; (*e*) selection differentials for the developmental stages and for imagoes ; (*f*) the total productivity of the population at any given time.

3. THE EXPERIMENT

In the experiment to be described here, four populations of *Drosophila melanogaster* were kept at 25° C., on the standard corn-meal, agar, molasses medium seeded with a constant amount of baker's yeast. Two of these (P_1 and P_2) were started about one year earlier than the other two (P_3 and P_4). The foundation stock of all four populations consisted of 10 virgin females and 10 males of wild-type Oregon-R strain, and 10 virgin females and 10 males of another strain homozygous for the sex linked genes *white* and *Bar*. The populations were bred in glass vials 12 cm. high and 3·5 cm. in diameter for about 100 generations over a period of four years. After

equilibrium was reached the population numbers fluctuated around 700 flies at first, but increased later. A total of about 2,000,000 flies were counted and classified for sex and phenotype.

Figs. 2 and 3 summarise the elimination of the mutant genes in competition with their wild-type alleles. As it will be seen, the mutants *white* and *Bar* were completely replaced by their normal alleles in the course of approximately 30 generations. The selection coefficients of the two genes were very similar throughout the experiment. As has been previously pointed out (Buzzati-Traverso, 1947), the selective value of these genes remains the same irrespective of its absolute frequency in the population. This conclusion is based on a plot of

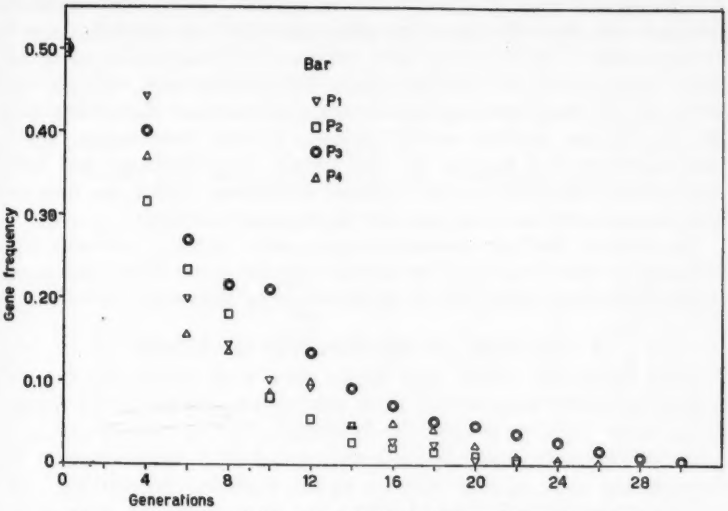

FIG. 3.—Elimination of the mutant *Bar* in the four experimental populations (P_1, P_2, P_3, P_4).

the logarithms of observed gene frequencies against time expressed in generations.

At their face value, the results obtained with the four populations showed no more than what might have been expected from similar data published by other authors. (L'Héritier and Teissier, 1934 and 1937; Reed and Reed, 1948 and 1950; Merrell, 1933*a* and *b*). The technique employed in this experiment, however, makes possible, as it has been pointed out before, to get more detailed information on the selection mechanism responsible for eliminating the mutant alleles. This aspect of the experiment will be analysed in a separate paper. Here only two facts brought out by the detailed analysis need to be mentioned in view of the discussion to follow: (1) the average generation time was 15 days; (2) the mean life of the adult flies in the experimental populations averaged 8·82 days.

Disregarding for the time being the obvious changes which occurred, when in thirty generations two genes, originally present in half the individuals, completely disappeared, one can still raise the question whether natural selection also brought about other genetic changes not so easily detectable but perhaps of even greater significance for adapting the fly populations to the conditions described. In other words, were the wild-type flies constituting the population after the disappearance of the mutant types genetically identical to the Oregon-R and *white-Bar* strains? These certainly differed from each other not only in the characters of the eye which made them easily distinguishable, but also in an indefinite number of genetic factors affecting other morphological and physiological characters. Now, did natural selection also affect this part of the genotype of the two original strains? Is it possible to find whether any genetic changes were produced in this respect and if so, of what sort? And, if any such changes did occur during the process of eliminating the *white* and *Bar* alleles, did the populations undergo similar changes in later generations, when their members had become all wild type? How different and how much better adapted to the cultural conditions might the flies be after natural selection had operated for many generations?

In order to find the answers to these and to similar questions, the populations were continued for another seventy generations, and were subjected to tests which will be analysed in the following sections.

4. CHANGES IN COMPONENTS OF FITNESS

The characters which most likely have been influenced by the process of natural selection are those directly responsible for the fitness of the flies. It is clear that the individuals having greater viability under the chosen experimental conditions had a better chance of contributing their genetic material to the following generations. In an experiment of this type viability can accordingly be defined in terms of relative survival value in competition. Various factors may contribute directly or indirectly to the production of a greater survival value, and it is possible to devise various tests to measure some of them. If we observe that one strain has a higher survival value than another, we may try to relate this difference to different values of some measurable characters such as fecundity, fertility, rate of development, etc. (see below). If such factors are inherited at all, one should be able to find whether their values in the experimental populations were greater than in the two original strains. It is known that in *Drosophila melanogaster* such characters are indeed heritable largely on the basis of a multifactorial or polygenic scheme. With this idea in mind samples of the two original strains and of the four populations were tested at different times. The following characters were measured: (*a*) percentage of sterile females; (*b*) fecundity of single females, measured by the number of eggs laid per day; (*c*) fertility, in the sense of Pearl (1932), *i.e.* the percentage of adults emerging from a

known number of eggs ; (*d*) rate of development, or the average time between the laying of the egg and the emergence of the imago ; (*e*) adult longevity, or the average time between emergence of the adult and its death.

(*a*) *Sterility*

Approximately 20-30 individuals of both sexes were taken at random from the Oregon-R, the *white-Bar* strains and from the four populations and were transferred to 300 c.c. culture bottles. Female offspring from these matings were cultured singly with three brothers each in 3·5 × 12 cm. vials. Every four days the flies were transferred to a new vial with fresh food, such transfer being repeated five times. If by the 24th day no larvæ were observed on the medium, the female was considered to be sterile. The number of sterile females was recorded, after due allowance for accidental deaths. Table 1 shows clearly that the incidence of sterile females in the original stocks was reduced to zero in the experimental populations. If one takes

TABLE 1

Sterility of females of original strains and of experimental populations

Strain or population	No. ♀♀ tested	No. ♀♀ sterile	Percentage sterility
Oregon-R	252	4	*1·58*
white-Bar	252	32	*12·69*
P_1	250	0	*0*
P_2	248	0	*0*
P_3	251	0	*0*
P_4	252	0	*0*

the average viability as measured in terms of sterility of the two parental strains as being equal 1·00, the viability of the four populations improved by a factor of 1·07.

(*b*) *Fecundity*

Samples of about 20-30 individuals of both sexes were taken at random from the two original strains and from the four populations and were cultured in 300 c.c. culture bottles (only in the case of tests performed in May 1948, see table 2, were the flies directly derived from the population bottles.) Virgin female progeny were isolated and mated singly within 24 hours from their emergence to three brothers in 3·5 × 12 cm. vials. The surface of the medium in these was covered with a disc of blue absorbent paper soaked in a suspension of yeast. The flies were transferred every day to a new vial of this type and the number of eggs laid on the blue paper during the previous 24 hours was recorded. Egg laying took place at 25° C., and the recording was continued to the 20th day of life of the female or to the time of her death. For each test and day the quantity and quality of food and laying medium was the same for both strains and all experimental populations.

Table 2 gives the results of various tests for fecundity at different times. Data are presented separately for the first ten days and for the first fifteen days of life of the females, because some of the females died before the fifteenth day. The mean life of flies in the populations being approximately nine days, it did not seem worthwhile to extend egg counts beyond the 15th day. Because of the laboriousness of egg counting over a period of several days, no counts were made for the two original strains in the May 1948 test. The relatively low figures for the four populations in this test are probably due to the fact that the females were sampled directly from the population bottles. They

TABLE 2

Fecundity of females of original strains and of experimental populations at various times

Strain or population	Date	Generation tested	10-day period		15-day period		Total no. eggs
			No. ♀♀ tested	Mean no. eggs/day	No. ♀♀ tested	Mean no. eggs/day	
Oregon-R .	2/48	...	6	*11·50*	4	*10·50*	1330
	1/49	...	13	*11·74*	10	*13·58*	2683
w-B . .	2/48	...	6	*6·45*	6	*9·53*	1107
	1/49	...	11	*15·00*	9	*16·63*	2397
P_1 . .	2/48	59	6	*32·45*	6	*29·46*	4297
	5/48	65	11	*22·34**	11	*29·85**	5940
	1/49	82	12	*37·36*	12	*35·70*	7748
P_2 . .	2/48	59	6	*31·90*	6	*30·30*	5104
	5/48	65	12	*25·38**	11	*29·72**	6140
	1/49	82	12	*31·31*	12	*37·09*	6843
P_3 . .	2/48	32	6	*27·46*	6	*26·26*	4318
	5/48	38	11	*22·03**	11	*26·00**	4925
	1/49	56	12	*28·70*	12	*28·64*	6063
P_4 . .	2/48	32	6	*33·43*	6	*34·33*	6537
	5/48	38	12	*16·55**	11	*20·65**	4232
	1/49	56	12	*34·83*	12	*34·17*	7296

* All the values of the May 1948 test are systematically lower for reasons explained in text.

were thus therefore certainly less well fed than those in the other tests, a factor of significance in egg production, since it is known that culture conditions during larval development affect fecundity (Hadorn and Zeller, 1943). Table 3 shows the results of the analysis of variance of the square roots of the actual numbers of eggs counted. The comparison between the mean number of eggs of the two original strains and those of the four populations gives a value of F which is significant beyond the $P = 0{\cdot}01$ value. Differences between various samples of the same strain or population, on the other hand, are not significant, with the exception of the *white-Bar* strain in which the

average number of eggs per day changed from 9·53 to 16·63 in the course of about one year. Even though the means of the populations

TABLE 3

Analysis of variance of transformed (square root) values of egg production for 15 days

	February 1948				January 1949		
	DF	SSQ	MS		DF	SSQ	MS
Total	33	1039		Total	66	1797	
Populations	5	795	159	Populations	5	632	126·4
	28	244	8·71		57	1165	20·4
	F = 18·25**				F = 6·19**		
	Oregon-R				*white-Bar*		
	DF	SSQ	MS		DF	SSQ	MS
Total	13	167		Total	14	214	
Samples	1	10	10	Samples	1	120	120
	12	157	13		13	94	7·23
	F = < 1				F = 16·56**		
	Population 1				Population 2		
	DF	SSQ	MS		DF	SSQ	MS
Total	28	559		Total	28	891	
Samples	2	12	6	Samples	2	12	6
	26	547	21		26	879	33·8
	F = < 1				F = < 1		
	Population 3				Population 4		
	DF	SSQ	MS		DF	SSQ	MS
Total	28	330		Total	28	1078	
Samples	2	4	2	Samples	2	169	84·5
	26	326	12·5		26	909	34·9
	F = < 1				F = 2·42		

for the February 1948 and January 1949 tests (table 2) are markedly different, especially in the case of P1 and P2, their F values are not

significant due to the wide range in the number of eggs of different females of the same population. Fig. 4 gives the mean cumulative fecundity of the two strains and the four populations over a period of 15 days and brings out in a very clear way the considerable difference in fecundity at the time of the last test between the original strains and the experimental population, derived from them. In spite of the already mentioned lack of significance between samples within populations, and disregarding the May 1948 test there seems to have been a trend toward higher values in each population as the selection experiment proceeded. The May 1948 test had smaller values than

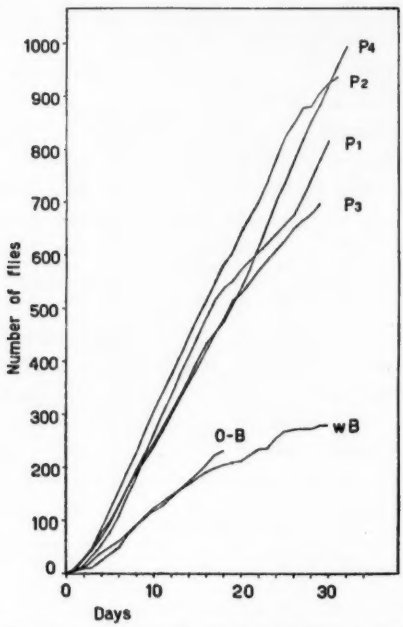

FIG. 4.—Comparison between the cumulative fecundities of the two original strains (O-B, *w-B*) and of the four experimental populations.

either of the others, but they were highly significant with respect to both tests on the original strains. Calculating the average means for Populations 1 and 2 on the one hand, and those of Populations 3 and 4 on the other, one can arrange the fecundity of females with respect to the number of generations as follows :

Generation	32	30·44	eggs per day
,,	56	31·76	,,
,,	59	32·17	,,
,,	82	37·33	,,

This pooling of data of the various populations seems justified by the remarkably parallel behaviour of the populations in every other respect, which makes the trend toward a continuous improvement in the fecundity very clear. If we take now the average viability as measured in terms of fecundity of the two parental strains at the first test as equal to 1·00, the average viability of Populations 1 and 2 at the 82nd generation had improved by a factor of 3·69.

(c) *Fertility*

Fertility, or the percentage of imagoes emerging from a known number of eggs, is influenced by the age of the mother, as shown by Hadorn and Zeller (1943). As a consequence, it was not possible

TABLE 4

Fertility of original strains and of populations at various times during the selection experiment

	Oregon		*w-B*	
	Egg no.	Per cent. emergence	Egg no.	Per cent. emergence
June 1947 . . .	200	*61·5*	200	*66·5*
Nov. 1947 . . .	200	*62·0*	200	*50·0*
Jan. 1949 . . .	3320	*64·51*	3320	*60·18*
Mar. 1949 . . .	1800	*79·00*	2040	*61·42*

Date	Generation	P_1		P_2		P_3		P_4	
		Egg no.	Per cent. emergence	Egg no.	Per cent. emergence	Egg no.	Per cent. emergence	Egg no.	Per cent. emergence
4/48	35	...	...	...	...	200	*73·5*	200	*81·00*
6/47	40	200	*90*	...	...	...	...	...	...
1/48	51	200	*81·5*	200	*93·5*	...	...	...	...
12/48	55	...	...	...	...	2430	*88·35*	2430	*92·75*
3/49	60	...	...	...	...	1360	*84·26*	1320	*89·92*
12/48	81	2430	*88·97*	2430	*86·62*	...	...	...	...
2/49	86	1020	*91·17*	1400	*84·28*	...	...	...	...

to use egg samples taken directly from a strain or population because of the uncertain age of the females concerned. Young daughters of flies sampled from the Oregon-R and the *white-Bar* strains as well as from the experimental populations were mated to several brothers and used as egg producers for the determination of fertility. Two tests were performed in 1947, for the sole purpose of determining fertility. In this case, groups of 20 eggs were transferred to 3·5 × 12 cm. vials; after developing at 25° C. the number of adults present in the vials was recorded. For any particular test special care was taken to have cultural conditions of the six experimental series as homogeneous as possible. The two sets of data of the 1949 tests, on the

other hand, are taken from an experiment in which the main purpose was to ascertain the weight of emerging flies. For reasons to be discussed below, the number of eggs per vial had to be different in each strain and population. The numbers of eggs per vial for the January 1949 experiment are to be found in table 10; the numbers of eggs per vial in the March 1949 experiment were as follows: Oregon-R: 45; *w-B*: 51; Population 1: 34; Population 2: 35; Population 3: 34; Population 4: 33.

Table 4 shows the results of fertility measurements on a total of 26,900 eggs. While in the Oregon-R strain a noticeable improvement took place in the course of almost two years, the fertility of the *white-Bar* strain remained stationary. All of the populations tested were clearly superior to both parental strains. No clear cut trend in the improvement in fertility with the progress of the selection is noticeable. This, however, can be related to the fact that the conditions of measuring

TABLE 5

Analysis of variance of fertility data. Transformed $p = sin^2 \phi$. *Data of June and November 1947 and of March 1949*

	d.f.	Sum of squares	Mean squares
Total	31	2218	...
Tests	2	365	182·5**
Populations	5	1532	306·4**
Error	*24*	*321*	*13·37*

fertility were not identical in successive tests. In order to test the significance of differences in fertility, two separate analyses of variance were made on the transformed values. The first (table 5) refers to the data of June and November 1947 and of March 1949, derived from vials not dissimilar in the number of eggs; the second (table 6) to the January 1949 data, for which a preliminary analysis of covariance had to be made in order to eliminate variability due to considerable differences in egg densities within each strain or population. In every case the data have been transformed to the sin^2 of the observed percentage. This statistical treatment confirmed the conclusions already noted regarding the differences between the fertilities of the two strains and of the four populations. If we now take the average viability as measured in terms of fertility of the two original strains at their first test as equal to 1·00, the average viability of the four populations at their last test has improved by a factor of 1·37.

(*d*) *Developmental rate*

Groups of 10 females and 20 males taken from strains and populations were transferred to 300 c.c. bottles having a paper spoon medium heavily seeded with baker's yeast. The size of the spoon was such

that it could be easily removed from and introduced back into the bottle without disturbing the flies. Collection of eggs for determining the developmental rate was made only on the second day after the flies had been transferred to the egg laying vials when the females had recovered from the shock of transfer and were laying eggs regularly. This technique made certain that the fertilised eggs had not stayed for a long period in the oviduct, and had therefore just started their development at the time of laying. The eggs were collected every four hours and transferred in groups of 20 into vials with equal amounts of food. The collecting took place at the same time for the strains and populations in order to insure the maximum of homogeneity in feeding and environmental conditions. Development took place at $25° \pm 0{\cdot}5°C$. After pupæ had been formed in the vials they were

TABLE 6

Analysis of covariance and test of significance of differences between adjusted means in January 1949 data of fertility. Transformed values $p = \sin^2 \phi$

Source of variation	Sums of squares and products				Errors of estimate		
	d.f.	Sx^2	Sxy	Sy^2	Sum of squares	d.f.	Mean square
Total . .	35	1,280,889	240,336	67,769	22,676	34	...
Populations .	5	176,002	236,344	67,341			
Error . .	*30*	*1,104,867*	*4,092*	*428*	*413*	*29*	*14·2*
					22,263	5	4452

For test of significance of adjusted means

$$Sy^2 - \frac{(Sxy)^2}{Sx^2} = 413 \qquad F = 4452/14{\cdot}2 = 313\ **$$

removed and placed singly in 30 c.c. glass test tubes containing a strip of filter paper which had been soaked in water to provide high humidity. Water was added each day to the test tubes until the flies emerged. Beginning with the seventh day after the eggs had been collected, the test tubes were inspected at 2.00 a.m., 8.00 a.m., noon, 4.00 p.m., 8.00 p.m. and midnight, and the number of emerged flies recorded. Thus the time of egg laying was known within ± 2 hours, and the time of emergence of the imago was determined within ± 1, 2 or 3 hours. The schedule chosen was not the best possible one because the time intervals were not constant, but in view of the large number of records to be kept at the same time for the six experimental series, no other choice was available. The test was performed on Populations 1 and 2 at their 53rd generation and on Populations 3 and 4 at their 27th generation. A total of 862 flies was used. The mean times of development expressed in days and hours, together

with the number of flies in each sample are given in table 7. In view of the fact that the time intervals were not constant no standard errors are given. In order to reach a more complete evaluation of the data, they are presented in graphical form in fig. 5, with time intervals of one day.

TABLE 7

Average time of development of samples of flies taken from the two strains and the four populations

Strain or population	Days	Hours	No. of flies
Oregon-R	10	6	123
white-Bar	9	11	100
Population no. 1	9	0	163
Population no. 2	8	19	170
Population no. 3	8	19	147
Population no. 4	9	12	159

A comparison of the distributions of developmental times of the four populations with those of the two strains indicates that the former are skewed towards lower values and that this is more marked in the

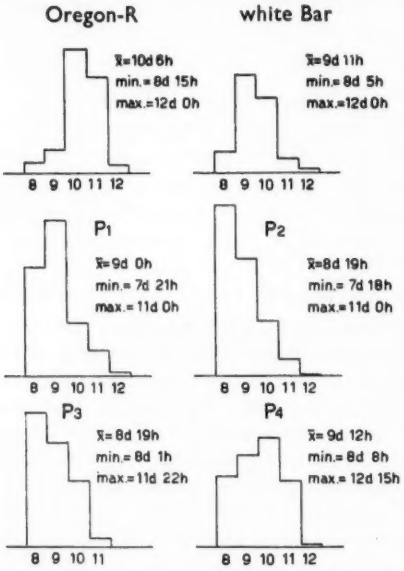

FIG. 5.—Distributions of the times of development for the two original strains and for the four populations. On the abscissa the days, on the ordinates the number of flies.

older populations (1 and 2) than in the younger ones (3 and 4). Furthermore, it is worthwhile pointing out that the extreme values of the distribution ranges shown on the figure are smaller in three

out of four populations. A comparison between the six distributions carried out with the chi-square test shows that they are highly statistically different ($\chi^2 = 245{\cdot}91$; d.f. $= 23$; $P \ll 0{\cdot}001$). Also, a chi-square test between the pooled values of the two original strains on the one hand and of the four populations on the other hand shows that they are different ($\chi^2 = 109{\cdot}76$; d.f. $= 7$; $P \ll 0{\cdot}001$), and even a comparison of the developmental rates of the fastest growing of the two strains, *white-Bar*, with the population having the skewest distribution, P_2, shows that the lower values of the latter are significantly different from the former ($\chi^2 = 50{\cdot}39$; d.f. $= 7$; $P \ll 0{\cdot}001$). It therefore seems fair to conclude that in the experimental populations natural selection favoured those genotypes which led to a faster rate of development. If one compares the mean time of development of the two original strains (9 days, 20½ hours) with that of the four

TABLE 8

Mean life of adult flies (in days)

	Oregon	*w-B*	P_1	P_2	P_3	P_4
No. of flies .	466	473	443	464	462	448
Average life .	*30·37*	*22·50*	*36·90*	*30·24*	*37·81*	*40·41*

	Analysis of variance		
	DF	SS	MS
Total . . .	2755	506,025	
Populations . .	5	98,278	19,655
	2750	*407,747*	*148*
		F = 132·5**	

populations (9 days, 3 hours), one finds a difference of 17·5 hours, or about 7·4 per cent. of the whole period of development. Thus, assuming that under the optimal conditions of this test time of development equals generation length, it is possible to compute that over a period of 100 days the two original strains would have produced 10·15 generations as against 10·96 generations produced by the average population. Thus, if we take the average viability as measured in terms of developmental rate of the two strains as equal to 1·00, the average viability of the four populations at the time of the test has improved by a factor of 1·08.

(e) *Adult longevity*

Ten groups of 50 newly emerged flies which were the progeny of samples taken from the strains and populations were placed in

3·5 × 12 cm. vials with standard food. They were transferred every five days without etherisation into a new vial with fresh food and record was kept of the deaths which had occurred during the interval. The flies were kept at 25° ± 0·5° C. throughout the experiment.

Populations 1 and 2 had reached the 73rd generations, and Populations 3 and 4 had reached the 47th when the samples were taken. Table 8 summarises the observed results. The actual numbers of flies of each group were somewhat less than the original 500 because some escaped in the process of transfer and some died of accident. The length of life has a normal distribution and the analysis of variance can accordingly be calculated on the actual figures. The differences between the observed means are highly significant, as shown in the table. Populations 1, 3 and 4 show mean life length values which are considerably greater than either of the two parental strains; only Population 2 has a length of life equal to that of the Oregon-R strain.

As has been noted earlier, the mean life of the flies in the population under experimental conditions was about nine days. It seems hard to explain how a life span beyond the 22nd or 30th day under optimal environment could be of advantage under conditions of very great overcrowding. The data concerning fecundity and longevity in *Drosophila melanogaster* appear to be contradictory. Alpatov (1932) found that they are negatively correlated, and Hadorn and Zeller (1943) found that they are positively correlated. Our data indicate that there is no particular parallelism between them. Thus, it may be that they are products of independent genetic and physiological processes.

Longevity does not therefore appear to be selected for either directly or as a correlated response of the improvement in fecundity. It seems likely that it is the product of some general factor for increased resistance and hardiness which has been favoured during the period of selection. If this is the case, and if we take the average viability as measured in terms of longevity of the two original strains as equal to 1·00, the average viability of the four populations improved by a factor of 1·37.

5. CHANGE IN MORPHOLOGICAL CHARACTERS

The evidence so far discussed indicates that natural selection has operated at the level of quantitative physiological characters and that the genotypes of the four populations after several generations differ from those of the two parental strains. In view of these facts a new question can be raised: do these quantitative physiological changes find a counterpart in some quantitative change at the morphological level? If this is the case, we might understand better the changes in size and shape which have occurred in many organisms in the course of evolution. Two sets of experiments were started in order to bring some evidence on this point; the object of the first was to ascertain whether any changes had occurred in the size

and shape of the wing, and of the second to see whether the body weight and size had changed appreciably.

(*a*) *Wing size*

In order to make sure that larval competition did not influence the size of the characters to be measured, batches of 20 eggs, obtained from females hatched from samples taken from the strains and populations, were placed in $3{\cdot}5 \times 12$ cm. vials with a constant amount of food and kept at $25° \pm 0{\cdot}5°$ C. The left wings of about 50 males were removed and dry mounted between two glass slides. They were then enlarged 133 times by projection with a Bush Metaphot microscope and measured on millimetre paper.

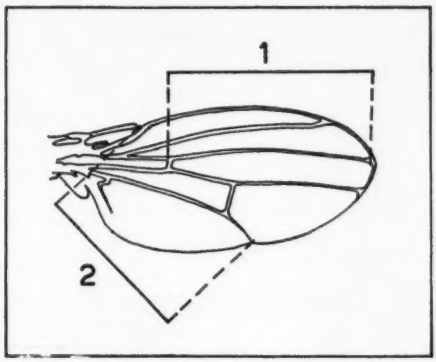

FIG. 6.—The two characters measured on the wings of the flies.

Table 9 records the results of the measurements and of the analysis of variance. The differences between the six means are highly significant, and the values of t for the differences between means taken two at a time show that (*a*) Oregon-R has wings longer than those of any other group; the statistical significance is at the 0·05 level for Population 4 and at the 0·01 level for the other four; (*b*) only Populations 2 and 3 have wings shorter than the *white-Bar* strain; (*c*) Oregon-R and *white-Bar* both have wider wings than any of the populations, but only Populations 2 and 4 show a statistically significant difference from the latter strain.

On the basis of such data we reach the general conclusion that under the experimental conditions genotypes with shorter and narrower wings were favoured and that the intensity of size decrease does not seem to be strictly correlated with the number of generations. Smaller wings were probably advantageous for the flies which lived under the more crowded conditions of the populations but the response to selection was not equally rapid in parallel populations.

(b) *Body weight*

It seemed particularly interesting to find out whether the action of natural selection for several generations had had any effect on body size. To measure this with satisfactory precision in *Drosophila melanogaster* is difficult, but an indirect estimate could be obtained from body weight.

In order to make significant comparisons between the body weights of samples of adult flies derived from the strains and populations it seemed necessary to make the feeding conditions as similar

TABLE 9

Values of wing characters no. 1 and 2 (wing length and width)

Strain	No. wings	$\bar{x} \pm e_{\bar{x}}$	Analysis of variance
Character no. 1 (wing length)			
+Oregon-R	37	163·6±0·594	F = 6·38**
w-B	44	159·8±0·787	d.f. = 270 and 5
P_1	45	161·0±1·012	
P_2	50	157·7±0·726	$t_{.05}$ = 1·720
P_3	50	158·0±0·903	$t_{.01}$ = 2·268
P_4	50	161·6±1·126	
Character no. 2 (wing width)			
+Oregon-R	37	123·7±0·575	
w-B	44	118·8±0·571	F = 28·55**
P_1	45	118·0±0·706	
P_2	50	117·3±0·584	
P_3	50	116·7±0·828	$t_{.05}$ = 1·108
P_4	50	117·9±0·592	$t_{.01}$ = 1·146

Wing length			Wing width		
Oregon	163·6	* **	Oregon	123·7	**
P_4	161·6		*w-B*	118·8	
P_1	161·0		P_1	118·0	**
w-B	159·8	**	P_4	117·9	
P_3	158·0		P_2	117·3	
P_2	157·7		P_3	116·7	

as possible. In view of the different degrees of fertility shown by the strains and populations (see section 4*c*) it did not seem valid to let equal numbers of eggs develop on a constant amount of food. In order to take this factor into account, a first experiment was carried out, by using six different egg densities for each strain and population. The actual densities were calculated on the basis of the fertility data in such a way as to produce similar larval and pupal densities. It was hoped that by this procedure one could perform the analysis of covariance of the observed data, and calculate the F on the adjusted

means after elimination of the density variable. The general results, which can be seen in table 10, however, were not suitable for this treatment, because it turned out that the regression between egg density and weight was linear for the Oregon-R and the *white-Bar*

TABLE 10

Mean living and dry weight of samples of 100 adults (50 ♀♀ and 50 ♂♂) expressed in 10^{-3} gr., hatching from different egg densities (indicated in parentheses)

	Oregon	*white-Bar*	P_1	P_2	P_3	P_4
Adults and eggs per vial	256 (40)	160 (40)	204 (30)	217 (30)	212 (30)	213 (30)
Samples weighed	2	1	2	2	2	2
Mean living weight ($\bar{x}$)	9·19	9·79	11·42	11·78	11·63	11·14
Mean dry weight ($\bar{x}$)	*2·48*	*2·56*	*2·91*	*3·17*	*3·02*	*2·92*
Adults and eggs per vial	240 (120)	200 (120)	246 (90)	230 (90)	244 (90)	241 (90)
Samples weighed	2	2	2	2	2	2
Mean living weight ($\bar{x}$)	8·75	9·23	8·45	8·45	9·52	8·82
Mean dry weight ($\bar{x}$)	*2·37*	*2·52*	*2·19*	*2·30*	*2·68*	*2·41*
Adults and eggs per vial	261 (200)	266 (200)	260 (150)	244 (150)	259 (150)	282 (150)
Samples weighed	2	2	2	2	2	2
Mean living weight ($\bar{x}$)	7·46	8·30	7·80	7·92	8·70	7·70
Mean dry weight ($\bar{x}$)	*1·85*	*2·31*	*2·10*	*2·17*	*2·41*	*1·95*
Adults and eggs per vial	411 (300)	361 (300)	382 (210)	344 (210)	395 (210)	392 (210)
Samples weighed	3	2	3	3	3	3
Mean living weight ($\bar{x}$)	6·47	7·27	6·72	7·33	7·38	7·54
Mean dry weight ($\bar{x}$)	*1·86*	*2·08*	*1·78*	*1·88*	*1·97*	*1·96*
Adults and eggs per vial	463 (370)	459 (370)	482 (270)	477 (270)	472 (270)	509 (270)
Samples weighed	4	4	4	4	4	4
Mean living weight ($\bar{x}$)	6·66	7·82	8·36	8·17	8·24	8·09
Mean dry weight ($\bar{x}$)	*1·75*	*2·35*	*2·55*	*2·52*	*2·52*	*2·58*
Adults and eggs per vial	511 (450)	552 (450)	588 (330)	593 (330)	565 (330)	617 (330)
Samples weighed	5	5	5	5	5	5
Mean living weight ($\bar{x}$)	5·70	7·14	6·68	6·71	6·93	6·72
Mean dry weight ($\bar{x}$)	*1·55*	*1·95*	*1·71*	*1·77*	*1·74*	*1·68*
Total adults	933	903	963	931	951	1005
Mean living weight (100 flies)	7·371	8·258	8·201	8·393	8·733	8·335
Mean dry weight (100 flies)	*1·976*	*2·295*	*2·206*	*2·302*	*2·381*	*2·250*

strains, but not for the four populations. If a transformation was used for all the values which made the regression of the populations linear, then the regression of the two strains became curvilinear. From a direct inspection of the data, however, it appears again that every population gave weight values which were greater than those of the Oregon-R strain and equal or slightly superior to those of the *white-Bar* stock.

In view of this difficulty, a second experiment was carried out using just one egg density per strain or population, as indicated by the numbers in parentheses in table 11 (experiment 2), which also summarises the data of the first experiment. A total of 8940 eggs was used, and besides weighing the sample groups of males and 50 females and comparing their mean weights, the total weight of adults produced in an average culture of each strain and population was calculated, by multiplying the mean weight by the actual mean number of adults obtained per culture. The two sets of data are in good agreement, in that they both show that the flies derived from

TABLE 11

Weight of Drosophila expressed in 10^{-2} gr. For explanation see text

	Oregon-R	*white-Bar*	P_1	P_2	P_3	P_4
Experiment 1						
Mean weight (100 flies)—						
Fresh	7·371	8·258	8·201	8·393	8·733	8·335
Dry	*1·976*	*2·295*	*2·206*	*2·302*	*2·381*	*2·250*
Total weight—						
Fresh	4·127	4·743	4·739	4·688	4·983	5·026
Dry	*1·106*	*1·243*	*1·275*	*1·285*	*1·359*	*1·357*
Experiment 2						
Mean weight (100 flies)—	(45)	(51)	(34)	(35)	(34)	(33)
Fresh	11·111	12·550	12·585	12·841	12·533	12·344
Total weight—						
Fresh	*3·270*	*3·488*	*4·019*	*3·794*	*3·598*	*3·667*

	♀ Oregon-R × ♂ *white-Bar*	♀ *white-Bar* × ♂ Oregon-R
Mean weight (100 flies)	(33)	(33)
Fresh	12·643	12·688
Total weight—		
Fresh	3·709	3·869

the four populations have produced more "Drosophila material" per culture than flies obtained from either of the parental strains. The actual values are lower in the second experiment than in the first because of the smaller number of eggs present in the culture vials. The data from the crosses between the strains in table 11 will be considered later.

It seems therefore safe to conclude that under the experimental conditions those genotypes were favoured that allowed the flies to make a better use of the available food, assimilating it and transforming it into "Drosophila material," and thereby reaching a greater weight and thus, presumably, a larger size.

6. THE NEW GENOTYPE

The evidence thus far presented shows that in the course of these experiments every tested character in each of the four populations

has undergone a certain change. The degree of change was different in each population, but the trend was the same in all. For some characters, as was the case for the fecundity of females, the values finally reached were much higher than those of either of the two parental strains, while in others, such as wing length, they were very close to one or the other of the two original stocks. Furthermore it has been shown that these changes continued after the mutant genes had disappeared, and the members of the populations all had a wild-type phenotype.

The physiological characters tested can be regarded as measures of fitness and because of this consistent change toward a greater total fitness the inference seems justified that the observed changes clearly had a selective value and were the product of natural selection. The viability factors which were calculated with respect to the average values of both parental strains for each tested character, and were presented at the close of subsections of section 4, can be used to estimate the total improvement in fitness of the populations. If the factors computed for sterility, fecundity, fertility and developmental rate are multiplied together one gets a compound factor of 5·84. And if the improvement in longevity can also be considered, as an index of an increase in viability, then the total factor of fitness increase becomes equal to 8·00. In other words, the total fitness improved in the course of about 80 generations by about 800 per cent. This value may represent a potential which would not actually be realised in the population bottles because all of the measurements were made on flies which developed under optimal conditions, which certainly did not exist in the population bottles. But since the improvement in each of the tested factors was certainly of an hereditary nature, the factor of 8 may be viewed as a valid estimate of the amount of evolutionary change in fitness between the beginning and the end of the experiment. The change in wing size was also probably adaptive, since it can be surmised that under conditions of extreme overcrowding such as prevailed in the population bottles, smaller wings must have favoured survival. It is not as easy, at first, to account for the associated increase of body weight and therefore presumably of size, because smaller individuals might be expected to have a better chance of survival in competition, under conditions of great density. But this change was accompanied by, or was the product of, a better ability of the flies to exploit the available food and to convert it into "Drosophila material." In view of this fact, one is led to conclude that the increase in weight, too, had a positive selective value. We shall discuss its significance later.

It would appear, therefore, that new polygenic systems have arisen from the genotypes of the two original strains which have conferred on their carriers a remarkably higher viability and have thus increased substantially the fitness of the populations to the conditions in which they existed.

That new and better adapted genotypes were produced seems beyond any possible doubt, but they need not necessarily have been the product of a long continued selection. If this had been operating on the products of genetic recombination resulting from the mixture of the two sets of genes present in the Oregon-R and the *white-Bar* strains, in each of the four populations a new gene constellation would have been synthesised through a progressive process of casting off the less adapted and preserving the most favourable polygenic combinations. But the observed higher adaptive level could have been the result of a different process, namely that complementary-acting genes were brought into combination at the F_1 and stayed in the populations without selection at the F_1 gametic frequency, plus or minus sampling fluctuations. In this latter case the higher fitness

TABLE 12

Heterosis in the crosses between the two original strains

Fecundity of females					
Cross	No. ♀♀ tested	Mean 10 d	No. eggs/day 15 d	Total 10 d	No. eggs 15 d
♀ *w-B*×♂ +	12	49·67	*59·93*	5961	8942
♀ + ×♂ *w-B*	12	39·05	*40·54*	4686	7229

Fertility			
Cross	No. eggs	No. adults	Percentage
♀ *w-B*×♂ +	1000	906	*90·6*
♀ + ×♂ *w-B*	1000	900	*90·0*

of the populations would have been produced by a sort of heterotic response due to the complementary action of the genes, and it would have been achieved in one step, at the first generation, rather than through a steady progress extending over several generations. Viability factors such as those measured in this investigation do in fact often show heterosis when two unrelated strains are crossed.

Two tests on viability factors and one on body weight were accordingly performed on the two reciprocal F_1 hybrids of the original strains to see whether this alternative interpretation was plausible. The pertinent data were collected following the previously described techniques. As can be seen in tables 11 and 12, the hybrid values show a clear-cut heterosis reaching values similar and sometimes higher than those of the samples derived from the populations after many generations. This being the case, the previously discussed

interpretation attributing the higher adaptive level of the populations to the action of a long continued selection should be rejected.

7. COMPETITIVE ABILITY OF THE NEW GENOTYPE

If indeed no selection for quantitative traits had occurred and the populations had just been maintained in hybrid condition over the whole experiment, other questions arise. Thus, in view of the fact that, except for the May 1948 test for fecundity, all the measurements of viability factors and of morphological traits were performed on the progenies of samples taken from the populations, and were cultured under optimal conditions allowing both heterotic and non-heterotic individuals to survive, how could the reported higher fitness obtain? Similarly, how could the indication of progressive change in fecundity be explained? No wing measurements were performed on the heterotic hybrids, but it was hard to believe that they could have had wings smaller than the parental strains, as it is known that wing length is positively correlated with body size (Zarapkin, 1934; Buzzati-Traverso, 1947*b*). This being true, how could the data presented in table 9 be accounted for? And, finally, how could the increase in population size to be discussed in the following section (8) be expounded?

Because of these questions an experiment was required which might discriminate between the two alternative interpretations and explain the causes of the observed changes. If the observed change in viability had been due to the effects of natural selection, which had produced a new genotype better adapted to the culture conditions, it follows as a necessary consequence that this new combination of genes would have a better survival value in population bottles than strains which had not been subjected to similarly stringent selection. This supposition could be tested if the wild-type flies derived from the experimental populations were put in competition with the original *white-Bar* strain, using the same technique of breeding that had been followed previously. Should the greater viability be the result of a new combination of genes selected over many generations then one would expect that the rate of elimination of the *Bar* and *white* alleles would be faster than in the four original populations. If, on the contrary, the greater viability was due to some sort of complementary action of genes maintained at their F_1 frequency, then no such difference between the results of the old and the new selection experiments could be observed. Two new populations, referred to as Selected 1 and 2 (PS1, PS2), were therefore started with 10 virgin females and 10 males taken from Population 1, and 10 virgin females and 10 males taken from the *white-Bar* strain.

Table 13 and fig. 7 give the results obtained in the course of the first fifteen generations. The comparison with similar data of the four original populations shows beyond doubt that in the Selected populations the rate of elimination of the *white* and *Bar* alleles was

much greater than in the first experiment. After 15 generations the mutant alleles were still present with a frequency of about 15 per cent. in the four old populations, but had already practically disappeared in the new ones. These data seem therefore sufficient to warrant the interpretation that the change in physiological and morphological characters was due to the action of natural selection which in the

TABLE 13

Selection rate in two of the original four populations and in the two new (Selected) populations

Generation	P_3		P_4		PS1		PS2	
	q_B	q_w	q_B	q_w	q_B	q_w	q_B	q_w
1	0·500	0·500	0·500	0·500	0·500	0·500	0·500	0·500
3	0·625	0·625	0·330	0·340	0·132	0·132	0·031	0·031
5	0·399	0·366	0·383	0·390	0·043	0·043	0·030	0·022
7	0·276	0·352	0·163	0·179	0·014	0·029	0·014	0·032
9	0·242	0·283	0·181	0·179	0·023	0·025	0·049	0·041
12	0·201	0·229	0·130	0·160	0·015	0·027	0·029	0·018
15	0·148	0·156	0·080	0·117	0·027	0·034	0·035	...

course of several generations has produced from the combination of the two original genotypes new polygenic constellations which ensured a better viability to their carriers and thereby made the populations progressively better adapted to their environments.

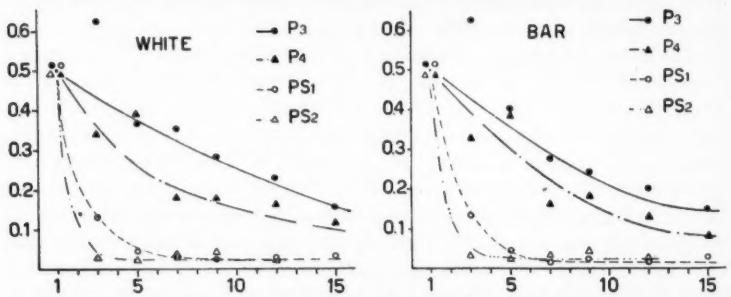

FIG. 7.—Elimination of the mutants *white* and *Bar* in Populations 3 and 4 and in the two Selected Populations. On the abscissa the number of generations, on the ordinate the gene frequencies.

8. NATURAL SELECTION MECHANISMS IN EXPERIMENTAL POPULATIONS

The growth of a population, whether natural or experimental, is known to be the result of the interaction of two groups of factors; the "biotic potential" of the species and the "environmental resistance" (Chapman, 1928).

"The biotic potential represents the potential rate of increase of the species under given conditions. It is realised if there are no restrictions of food, no toxic waste products, etc. Environmental resistance can be measured by the difference between the potential number of organisms which can appear during a fixed time in consequence of the potential rate of increase, and the actual number of organisms observed in a given microcosm at a determined time. Environmental resistance is thus expressed in terms of the reduction of some potential rate of increase, characteristic for the given organism under given conditions" (Gause, 1934).

The increase in number of organisms per unit time depends in the first place on the potential number of offspring (b) which the organism can produce per unit time; the total potential number of organisms present at a given time in a given environment will be given by the product of such potential increase by the number (N) of organisms present at the time considered. If there were unlimited food supply and no other limiting factors the population would grow according to an equation of geometric increase, where the constant b is the rate of potential increase of the organism. Under experimental conditions, as well as in nature, food is limited and other factors may further interfere with the growth of the population; therefore the potential geometrical rate of population growth is not realised, and its limitation is due to a complex of factors which are described under the general term "environmental resistance." In a limited environment with a constant supply of food, such as our population bottles, the potential geometric increase at every moment will be only partially realised, depending on how near the already existing size of the population reaches the maximum number (K) of individuals that can live within the limits of the chosen biotope or its numerical equilibrium. The difference between the maximal population possible and that already realised, expressed in terms relative to the maximal population, shows the number of "still vacant spaces" at a given time in a given microcosm (K-N/K). In accordance with this formulation, in populations like the ones of *Drosophila melanogaster* here considered, which were started with a number of adults much smaller than that of the population at numerical equilibrium:

(1) The rate of growth will increase rapidly at first, will reach a maximum and then decline until attainment of the numerical equilibrium.

(2) The environmental resistance will be very small at the beginning and then will increase to a constant maximum value when the population has reached its maximal number, and correspondingly:

(3) The intensity of the struggle for existence, measurable by the ratio of the unrealised part of the potential increase over the realised part of the potential increase, is very little at the beginning of the experiment, but later increases considerably and very rapidly.

The quantitative treatment of the interaction between the biotic potential and the environmental resistance has been expressed by

Verhulst and by Pearl and Reed by means of the logistic curve: $\frac{dN}{dt} = bN\frac{K-N}{K}$. A general discussion of this conception can be found in Gause (1934).

Pearl and his co-workers (see Pearl, 1927) demonstrated that the growth of genetically homogeneous populations of *Drosophila melanogaster* follows the logistic curve. This interpretation is borne out by the data of L'Héritier and Teissier (1933), Bodenheimer (1938) and of the writer so far as homogeneous populations are concerned. The experiments reported here, however, are concerned with populations

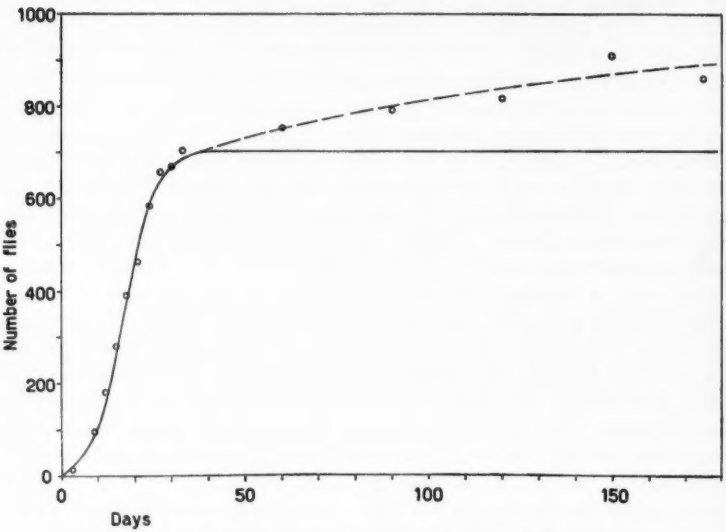

FIG. 8.—Comparison between the observed population growth (circles and broken line) and the calculated logistic curve (solid line).

which are changing genetically rather than with purely demographic phenomena. The experimental populations were started with equal numbers of two genotypes. While numerical equilibrium was being reached and later, a struggle for existence took place between the various genetic combinations present. The end result was a change in the biotic potential itself, which led to a further increase in the number of flies present in the bottles, beyond a first equilibrium value, as can be seen in fig. 8. This shows that the classical logistic curve is not realised in genetically heterogeneous populations. It accordingly seems worthwhile to analyse how this may occur.

The two groups of flies originally present in the first bottle differed in the shape and colour of the eyes and, we can now say, in fitness or biotic potential. This original population was accordingly genetically

heterogeneous. At the beginning there were two genotypes which had to face only a small environmental resistance. From the second generation on, however, the struggle for existence became much more complicated because of genetic recombination, which led to an increase in the number of genotypes present. How large this increase was is unknown. It must have produced phenotypic variability in the considered components of fitness beyond the range of variation of the original strains ; but more important for the present discussion, it must also have produced a range of biotic potentials sufficient to permit the operation of natural selection within the conditions prevailing in the bottles. This range must also have changed continuously during four years.

The ways in which different genotypes became represented by different average numbers in the next generation are certainly numerous and we can only attempt to list a few, with particular reference to the factors which have been measured in this investigation.

In the first place, adult flies having different genetic constitutions may show different percentages of sterility, as was the case for our Oregon-R and *white-Bar* stocks. In the second place, flies of one sex can be differentially attracted by flies of the opposite sex having other genotypes ; this seems to be at least in part the cause for the elimination of *white* gene reported by Reed and Reed (1950) and by Merrell (1953), who showed that both red- and white-eyed females discriminate against white males in mating. In the third place, flies genetically different can have different average fecundities, as was shown above. In the fourth place, selective fertilisation might occur. Certain types of sperm might fertilise certain types of eggs more easily than others ; there is no satisfactory experimental evidence on this point, but the possibility cannot be excluded. In the fifth place, different genotypes may differ in fertility as was shown above, and this in turn may depend on a series of factors such as presence of lethals or semilethals, the ability to grab food, capacity to assimilate it, etc. In the sixth place, developmental periods may be different, as shown above. In the seventh place, hardiness in the adult stage can be different, as was found in our experiments in terms of longevity. In the eighth place, resistance against diseases at various life stages can be different. Different values of each of these and similar factors will contribute to the differential success of any genotype in competition, provided that they are genetically determined and extend beyond the range of environmental variation.

While discussing the new adaptation level reached during the experiment (Section 6) it was pointed out that the total potential viability of the populations had increased by a factor of 8. The intensity of the struggle for existence under the experimental conditions employed can be evaluated by the ratio of the unrealised part of the potential increase to the realised part. The four populations reached their first numerical equilibrium after 33 days at an average value

of 704 flies per bottle. The integrated differential equation of the logistic curve best fitting the experimental data is :

$$\frac{704}{1+e^{4\cdot03-0\cdot2344t}}$$

From this expression the values shown in table 14 can be obtained. The lowermost figures of this table give an index of the struggle for existence. It will be noted that this has increased over 1000 times from the outset of the growth of the population to the 33rd day. Is it possible to evaluate the magnitude of competition in the population bottles in a more direct way? Using Pearl's data (1932) on the influence of density of population upon egg production in *Drosophila melanogaster*

TABLE 14

Growth of populations of Drosophila melanogaster. *The observed values are the average of the four parallel experiments.* b (*biotic potential*) = *0·2344*; K (*maximum population*) = *704*

Day	N observed	N calculated	$bN-\frac{dN}{dt}$
3	16	24·40	*0·036*
6	51	47·63	*0·073*
9	94	90·02	*0·141*
12	179	160·73	*0·297*
15	281	263·67	*0·600*
18	392	385·46	*1·212*
21	465	499·29	*2·448*
24	583	586·66	*5·024*
27	657	639·44	*9·989*
30	669	670·77	*20·278*
33	704	687·16	*42·481*

one can estimate the number of eggs actually laid in conditions like the ones prevailing in our experiment. Using this estimate and the average viability data presented above, one can compute the number of zygotes that did not reach the adult stage for every one that did. The figure thus obtained, 554, shows again how intense selective processes were under the experimental conditions.

It should be mentioned, furthermore, that with the progress of the experiment it was noticed that the four experimental populations did not remain at the original equilibrium of 700 flies but slowly approached and later exceeded the average number of 900 flies per bottle (fig. 8). At the same time it was noted that the number of adults emerging from each bottle was positively correlated with the number of individuals present in the population when eggs were being laid in that particular bottle. The correlation was curvilinear, as one would expect, in view of the fact that the maximum number of adults which can be recovered from a certain amount of food is practically constant beyond a certain number of fertilised eggs present in the original culture bottle. Buzzati-Traverso (1947*b*) has shown

that it is not possible to obtain more than an average of about 140 adult flies from between 300 and 1000 eggs in experiments in which the same type of vial and an inbred strain of *Drosophila melanogaster* were used and the eggs were placed on the medium all at the same time. It seems safe to conclude that the increased viability and ability to exploit the available food made the populations better adapted to the environmental condition in the sense that they became more numerous. If it were possible to calculate from our data the value of the biotic potential at the end of the experiment it is certain that we would find a figure larger than the original 0·2344.

The consequence of these results for problems of demography will not be discussed here (Buzzati-Traverso, 1954); but a conclusion seems ineluctable: that the intensity of natural selection increased during the experiment, even though the environmental conditions were constant. This conclusion pertains only to the action of natural selection on traits determined by polygenes, for no such increase in the intensity of the struggle for existence is considered by the current mathematical theories of evolution in which the effects of selection on the frequencies of genes primarily affecting a single trait have been analysed. A new theoretical treatment of natural selection in polygenic systems therefore becomes necessary.

One more comment seems pertinent. Most discussions of evolution from the standpoint of genetics consider the selective value of a certain gene, or chromosome type alone, and, so to say, *in vacuo*. The fact is that the change in frequency of a single gene (or chromosome) during a number of generations means that the individuals carrying its allele will produce more or fewer adult offspring in the next generation than the individuals not carrying it. The factor which is decisive for changing frequency is a *productivity differential* involving of necessity the whole genotype and not one gene alone, for the latter will have different survival values in different genetic milieus. Some extreme mutants, like those mostly used in laboratory experiments, may affect specifically the productivity of its carriers to such an extent as to make the effects of the rest of the genotype and of the interactions of the mutant with it insignificant. But under natural conditions the commonest case is very likely that of " small " mutants where the natural selection mechanism probably involves many genes at one time. Thus, a continuous increase in the intensity of natural selection fostering both productivity and change in polygenic traits may be of cardinal significance in evolutionary events.

Finally, I have shown that a given physiological or morphological trait may differ in degree in parallel experiments, and that, as a consequence, the improvement in fitness realised in the four populations described above was not identical after a certain number of generations. In view of the fact that we are dealing with characters which are controlled by many genes this is not surprising. The very exacting sieve of natural selection acts throughout the whole life cycle

of an organism and it is well possible that the same results, namely an increased total productivity or an adaptive change in morphological characters, can be reached in various ways through the action of various constellations of genes. For example, one of these may guarantee success during larval development, another may insure a hardier adult life. Each of two individuals carrying either of these constellations may show an identical total productivity value and yet the constituent factors, taken separately, can be very different. The complex genetic conditions which underlie the observed changes in fitness need not be the same in the four populations, nor indeed in all of their members. The only fact of significance is that one end result was achieved : higher productivity.

9. EVOLUTIONARY SIGNIFICANCE OF CHANGE IN POLYGENIC TRAITS

J. B. S. Haldane has pointed out (1949), that in view of the arbitrariness of distinctions between systematic categories, the most reliable measure of evolutionary rate is one which relates changes in the dimensions of the body of an organism or its parts, to time, whether expressed in units of geological time or in generations. It is generally accepted, however (see, *e.g.*, Fisher, 1930 ; Timoféeff-Ressovsky, 1939 ; and Simpson, 1944 and 1954) that mutations important for evolution are small, as measured in terms of structural change, and therefore that evolution can be explained in terms of the accumulation of small mutants affecting quantitative traits. This opinion seems substantiated by two considerations, that (*a*) differences between subspecies, species and higher systematic categories are ordinarily of a quantitative nature and accordingly fall under the control of polygenic systems, and that (*b*) a great majority of characters used by the taxonomist are in terms of measurements or ratios between measurements.

The data presented above bring evidence to show that evolutionary changes of the same degree as those observed by the paleontologist and the morphologist which may have taken millions of years to produce, can be observed within the limited span of a few dozens of generations. The variation in the size, and therefore shape, of the wings, as well as that concerning the size of the body are of this type. Furthermore, the correlation of reduction in wing size and increase in body size demonstrates that a change must also have occurred in the values of the coefficients of relative growth. These changes are significant for an understanding of evolutionary events not only from the viewpoint of qualitative differences but of quantitative ones also. If, in the case of the shortening of the wings, we take the values derived from Population No. 2 at its 55th generation ($157{\cdot}7 \pm 0{\cdot}726$) and compare it with that of the smaller of the two parental strains namely *white-Bar* ($159{\cdot}8 \pm 0{\cdot}787$), we can calculate

that the average rate of change per generation is 0·00024 per cent. This change corresponds to a little less than one-twenty-thousandth of the standard deviation of the value of Population 3. This means that the observed rate was of the same order of magnitude of the fastest evolutionary change, heretofore recorded, namely the length/height index of the human skull. This fact brings further evidence to indicate that known genetic processes are quite adequate to account for the evolutionary changes observed in the fossil record.

The analysis presented above brings still other evidence which may be of significance for an understanding of historical evolution. Paleontologists have found many examples of progressive increases in size within phyla. This seems to be a general trend among terrestrial vertebrates, and similar evidence is accumulating for other animal groups (see *e.g.* Stenzel, 1949). Castle (1932) argued that phylogenetic increases in size of this sort may be caused by competition among individuals in a given population of the same species and that the larger individuals or those attaining a large size earlier in life than others would be at an advantage over the smaller ones. Competitions *in utero* between mammalian embryos would be favoured by genetic conditions which would result in a more rapid rate of development, and thus a larger size at birth and maturity. It is quite possible that a similar phenomenon has actually occurred in our populations, where, as it has been shown, genotypes producing a faster development have been favoured.

My material also illustrates another evolutionary process. When the wild-type flies from the first natural selection experiment were put in competition with the original *white-Bar* strain, the two mutant alleles were eliminated at a much faster rate than in the four original populations, as a result of the greater fitness which had been attained by the " selected " wild type. Let us now consider a condition in nature in which there is a steady migration from Population A to Population B. As a result of this accretion of new variability by the latter, natural selection may produce genotypes better adapted to the environmental conditions of Population B by incorporating the favourable migrating genes and discarding the less favourable ones. In the course of time the pressure of selection against the latter would increase and they would accordingly be eliminated more and more rapidly, as was the case in our " new experiment " (see section 7). In this way a very elementary sort of isolating mechanism may arise.

New experimental data always raise new questions and broaden the scope of our investigations, and this attempt to analyse the action of natural selection on polygenic characters has accordingly proved to be quite rewarding. Its main significance lies in the fact that it may encourage the use of similar techniques in order to reach a deeper insight into the factors responsible for the " advancement of all organic beings, namely, multiply, vary, let the strongest live and the weakest die."

10. SUMMARY

1. Four parallel populations of *Drosophila melanogaster* were maintained in approximate numerical equilibrium for a period of about 4 years (100 generations) using Pearl's technique.

2. The foundation stock of each of the four populations consisted of equal numbers of males and females of a wild-type Oregon-R strain and of another strain homozygous for *white* and *Bar*. About 2,000,000 flies were classified for sex and phenotype. After about 30 generations the mutants were completely replaced by their normal alleles.

3. Samples of the two original strains and of the four populations were tested at different times for a series of physiological and morphological traits.

4. The following changes occurred in the experimental populations : (i) Sterility of females of the two original strains disappeared. (ii) A very marked and progressive increase in the average fecundity of females took place. (iii) The rate of development of the flies increased. (iv) The adult longevity of the flies increased. (v) Length and width of the wings become smaller. (vi) The average body weight of the flies increased.

5. The observed improvement in fitness by a factor of 8 over a period of about 80 generations as well as the morphological changes are interpreted as being due to the action of natural selection which, making use of the genotypes of the two original strains, has produced new polygenic systems better adapted to the conditions of the experiment.

6. To test the validity of this interpretation two new populations were started in which the wild-type flies derived from the previous experiment were put in competition with the original *white-Bar* strain. The rate of elimination of the mutant alleles was in this case much faster.

7. The mechanisms of natural selection in experimental populations are discussed and it is shown how the genetic heterogeneity of a population may affect its growth. This will follow a curve different from the logistic derived from the assumption of a constant value for the biotic potential of an organism which, on the contrary, falls under the control of natural selection. In fact the average number of adults in the four populations departed from the logistic curve calculated from a constant biotic potential. It is further concluded that the intensity of natural selection is bound to increase under constant environmental conditions if one takes into consideration polygenic systems.

8. The evolutionary significance of changes in polygenic traits is discussed. The observed rate of change in wing size is shown to be of the same order of magnitude as the fastest known rate of evolution as revealed by the fossil record. The increase in body size, too, is in agreement with progressive increase in the dimensions of animals observed by paleontologists for many phyla.

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THE TIME FACTOR IN P^{32}-INDUCED MUTATIONS IN MALE *DROSOPHILA*

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I. INTRODUCTION

FOLLOWING an earlier indication (Bateman and Sinclair, 1950) that the ratio of visible mutations to recessive lethals on the sex chromosome was higher for ingested Phosphorus-32 than for X-rays, the author

TABLE 1

Comparison of mutagenic action of P^{32} (50 μC per culture tube containing larvæ) and X-rays (4000 r to newly emerged adults)

Mutation	Remarks	Actual values		Percentages		Probability of difference
		P^{32}	X-rays	P^{32}	X-rays	
Dominant lethals * .	As indicated by fertility	...	...	...	...	...
	No. of daughters of Muller-5 ♀♀	4·7	10·3	87	71	...
	No. of sons of attached-*X y* ♀♀	13·6	20·5	43	13	...
Sex-linked recessive lethals	Muller-5 matings	11/130	13/199	8·5	6·5	0·5
Visibles . . .	,,	3/130	2/199	2·3	1·0	...
Deleted *X*'s . .	Non-*y* daughters from attached-*X y* ♀♀. All data	40/1520	15/1584	2·6	1·0	<0·001
	1st hatchings from 1st matings	18/693	2/735	2·6	0·3	<0·001
"Probable" visibles †	From attached-*X y* ♀♀	22/1520	16/1584	1·4	1·0	<0·3

* For dominant lethals "actual values" are fertility figures. "Percentages" are the percentage by which these fall below the control value; theoretically the percentage rate of dominant lethality.

† These are judged on phenotype alone since a very high proportion are sterile and cannot be tested genetically. The figures will therefore include some autosomal dominants and some non-heritable variants as well as genuine sex-linked recessives.

has made a direct comparison of the mutation spectra produced by the two mutagens. The mutation spectrum is partly dependent on dose and one should therefore endeavour to compare dosages of P^{32}

* The experiments on which this paper is based were carried out at the John Innes Horticultural Institution, Hertford.

and X-rays which give the same yield of a standard fraction, such as sex-linked recessive lethals.

In the first comparison (table 1) treated males were mated for three days, though some were re-mated for another three days. The P^{32} gave slightly more sex-linked lethals (not significantly so); an apparently higher rate of dominant lethals, measured indirectly from fertility; and a highly significant excess of hyperploidy due to supernumerary deleted *X*'s. The X-ray data were very heterogeneous in respect of this mutation. If we exclude the second matings and the later hatchings from the first, the discrepancy between the two sources of radiation becomes very striking: 2·6 per cent. for P^{32} as against 0·3 per cent. for X-rays. Finally there is a non-significantly higher incidence of "probable" visible mutants from the *y* attached-*X*

TABLE 2

Change of mutation rate with successive samples of sperm: P^{32} and X-rays compared

Days' mating	Dominant lethals * (per cent. eggs hatching)						Sex linked recessives (per cent. lethals + visibles)	
	P^{32}			X-rays			P^{32}	X-rays
	Series 1	Series 2	Series 3	Series 1	Series 2	Series 3	3 series combined †	
1	9·2	26·2	1·6	40·6	52·2	47·1	14·0	8·5
4	...	21·7	0 0	...	41·3	22·7	4·7	10·8
6 and 7	10·8	27·8	5·2	5·2	4·6	2·8	6·1	14·3
10	...	61·5	12·2	...	12·6	51·1	3 9	7·4
13	...	72·4	41·3	...	70·0	...	3·3	0·0
16	...	66·4	59·7	...	60·1	...	0·0	...

* Control value 70 per cent.

† The three P^{32} series are obviously heterogeneous, so that variation with time has little meaning.

matings. The high sterility of suspected mutant males means that one can make use of them only by guessing at their genetic nature. The total will thus include some autosomal dominants as well as some non-genetic variants.

The hyperploidy results indicated that comparisons of the mutagens would depend on the time of sampling of the sperm. So a second experiment was set up in which sperm was sampled every three or five days over an extended period (table 2).

It was at once apparent that the effect of mating sequence was quite different in the two treatments. X-rays produced a peak mutation rate for dominant lethals after the first few days, followed by a drop to the control value, as found by Lüning (1952) and Auerbach (1954). P^{32}, on the other hand, showed a constant rate for between

seven and ten days, after which it fell to the control level. There is a suggestion of the same effects with sex-linked recessives. Incidentally, pooling the data of the two experiments gives confirmation of a higher ratio of visibles to lethals with P^{32}. The ratios were 7 : 22 for P^{32} and 6 : 43 for X-rays, though the difference is not statistically significant ($P = 0{\cdot}2$). Only those visibles are included which are strictly comparable to the lethals in that they arose in the Muller-5 matings.

The experiment to be described investigates further, and analyses, the variation in mutation rate for one specific type of mutation, between successive sperm batches following the ingestion of P^{32} by larvæ.

2. FEEDING

The normal medium was made up with molasses, oatmeal and agar, which was then yeasted with a yeast suspension. The parents were added from mating tubes and shaken out again after 24 hours laying. Twenty-four hours later still, when all hatchable eggs had hatched and larvæ were in their first instar, P^{32} solution was added at 4 drops per tube from a calibrated syringe needle (4 drops : 0·03 ml.). The solution was placed well in the middle of the food surface to avoid seepage down between food and glass. Even then, however, there is considerable variation in activity between larvæ from the same tube, presumably owing to variation in activity over the food surface. When bottles were used the volume of P^{32} solution was increased five times to correspond to the increased surface area of the food.

It had been found that larger volumes of solution per tube tended to overflow between glass and food, causing an unreliable distribution of activity. If the volume was increased to as much as 0·5 ml. many larvæ were drowned. The dose per tube was 30 μC at the time of application (150 μC for bottles). Analysis of the food medium showed that most of the activity was in the top layer where the larvæ fed.

3. TYPE OF MUTATION

The " dominant lethal " method for studying mutation rates as used by many workers (*e.g.* Lüning, 1952) is rather insensitive owing to the variable proportion (usually 0-10 per cent. but sometimes much higher) of non-hatching eggs in the controls, and the ever-present danger of confusing dominant lethality with unfertilised eggs. An alternative method which gives the mutation rate in the first generation has been developed.

This involves scoring the frequency of non-*yellow* (hyperploid) daughters of *yellow* attached-*X* females mated to treated wild-type males. The sole source of confusion is the low spontaneous rate (about one in a thousand) of production of non-*yellow* daughters by " detachment " of the attached-*X*'s.* These are readily recognised by breeding tests. When the y^+-bearing fragment of the *X* is below a critical size the hyperploid females are fertile and their breeding behaviour is characteristic.

The advantages of the method are : the unambiguous recognition

* The author had not appreciated the frequency of detachment until the completion of the experiment so the above distinction was not made. Most of the non-*yellow* daughters after nine days' mating are probably the results of detachment.

of non-*yellow* females, even to an untrained observer ; control values practically zero, especially after elimination of detached-*X*'s ; and recognition in the first generation (though breeding for a further generation is necessary to detect the detached-*X* females).

The recovered hyperploids have arisen by breakage of the *X* chromosome in two places followed by rearrangement, which produces an acentric and a centric fragment. The centric fragment will almost certainly carry the y^+ allele which is very close to the left end. Females carrying fragments above a certain size die in the same way that triplo-*X* females die in normal attached-*X* matings. Very occasionally these " super-females " will be found in cultures, when they are recognised by their small size.

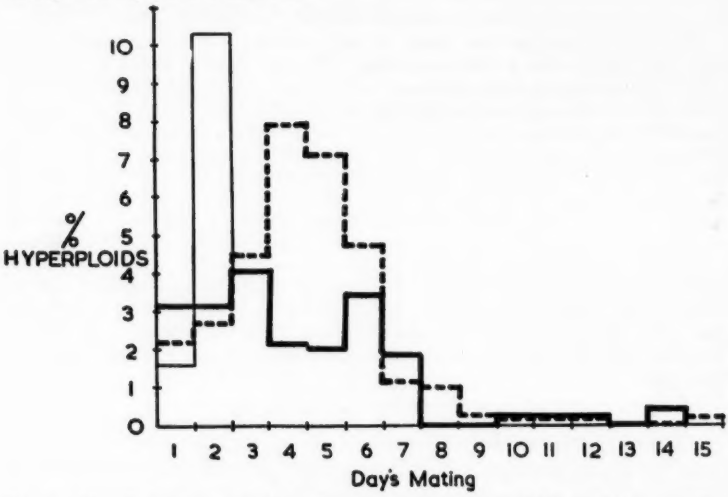


FIG. 1.—Mutation rate measured as the percentage of hyperploid daughters according to the day of mating in two series.

Entire line : ♂♂ pupating out of active medium.

Broken line : ♂♂ pupating in active medium.

Owing to the erratic value for the 2nd day of the first series (due to small size of sample) the weighted mean for the first two days is also shown. The difference between the two series is presumed to be the effect of external radiation on the pupa.

Hyperploids could also arise through *X*-*Y* or *X*-autosome translocations, but only following breakage in diploid cells.

The primary cause of hyperploids, chromosome breakage, is also responsible for the great majority of dominant lethals. Results based on hyperploidy should therefore parallel those expected from dominant lethal studies, only they will be more reliable. One important difference which must be borne in mind is that since hyperploids are dependent on a minimum of two breaks per chromosome, (two-event phenomena) their frequency will be more sensitive to variation in dosage or effective dosage (sensitivity times dosage).

4. OBSERVED RATE

The treated males consisted of two groups, those which pupated out of the active medium and were transferred on pupation to inactive tubes, and those which pupated in the medium. The internally generated radiation would be the same in both groups, but the external radiation would be greater and over a longer period in the second group. Unetherised males were mated singly when one day old to 5 *yellow* attached-*X* virgin females, also unetherised. Five fresh virgin unetherised females were supplied every day except for one two-day period from the 11th to 13th days. This high number of females was partly owing to the low mating frequency of females of this stock, but also to ensure that sperm was utilised as it matured.

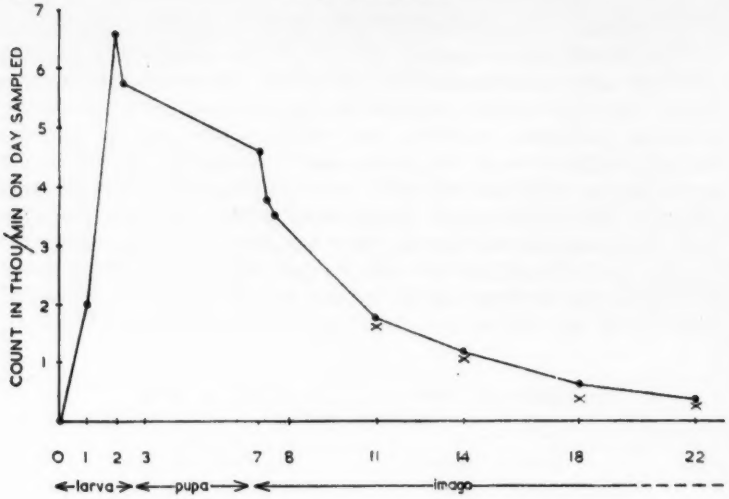
TABLE 3

Variation of hyperploid production with day of mating and site of pupation

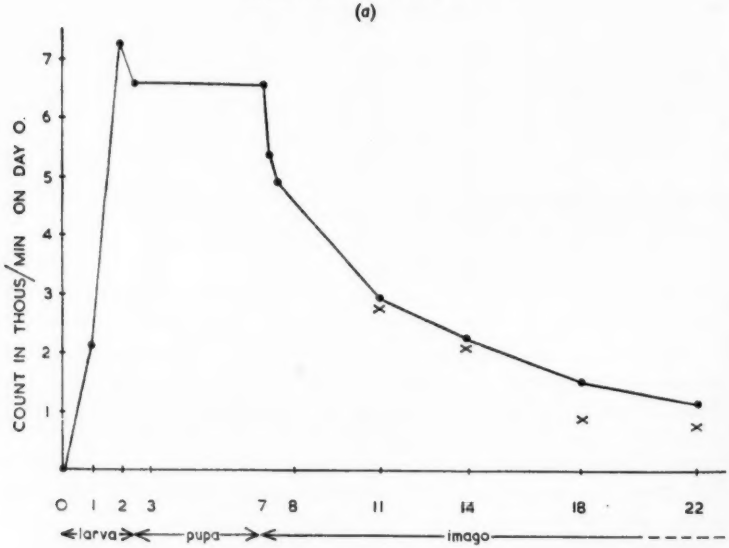
Day of mating	Pupæ out of medium			Pupæ in active medium		
	Non-*yellow* ♀♀	Total ♀♀	Per cent. non-*yellow*	Non-*yellow* ♀♀	Total ♀♀	Per cent. non-*yellow*
1	2	126	1·6	4	178	2·2
2	3	29	10·3	6	222	2·7
First two	5	155	3·2	...	...	...
3	6	149	4·0	8	180	4·4
4	1	46	2·2	6	77	7·8
5	3	148	2·0	5	70	7·1
6	4	116	3·4	11	236	4·7
7	4	225	1·8	2	174	1·1
8	0	337	0·0	8	785	1·0
9	0	260	0·0	4	1012	0·2
10	1	404	0·2	1	995	0·1
11	1	610	0·2	2	1463	0·1
13	0	540	0·0	0	784	0·0
14	1	252	0·4	0	572	0·0
15	0	185	0·0	1	825	0·1

The results are given in table 3 and fig. 1. By an unfortunate accident the formally necessary control matings were lost, but the incidence of non-*yellow* daughters from unirradiated matings is always very low. The effects of irradiation from the medium during pupation appear to be confined to three days, the 4th to 6th days' mating, but especially the 4th and 5th, although pupation extends over four days. During these two days the increase in mutation due to external radiation of the pupa is about equal to that due to all other sources.

The general shape of the curves in fig. 1 is not, however, quite as had been expected. Excluding the effects of external radiation of the pupæ, there is a gradual fall with each day's laying up to the 9th or 10th day. Yet the effect of P^{32} had been expected to be cumulative, the dose increasing continuously with the decay of the P^{32}. The explanation for this discrepancy follows.



(*a*)



(*b*)

FIG. 2.—Mean activity of larvæ, pupæ, and imagos of male *Drosophila* with successive, days from addition of P^{32} to the medium. Points joined by the line are for virgin males, crosses for mated males.

(*a*) Count in thousands per minute at time of sampling.
(*b*) Count as at the moment of addition of P^{32} (*i.e.* without radioactive decay).

5. VARIATION OF P^{32} CONTENT WITH TIME

The activity of larvæ, pupæ, newly emerged imagos and imagos aged on inactive medium were measured with a Geiger counter. The results shown in fig. 2 are the means for flies from three treatment bottles. Fig. 2*a* shows the activity on the date sampled in thousands per minute, and 2*b* the counts as they would have been at a constant time, the date on which the P^{32} was added to the medium. Thus the variation shown in fig. 2*b* is exclusive of radioactive decay. Mostly it is due to excretion. There is an additional loss from mated males which is almost certainly due to the phosphorus transferred in the sperm. Counts of females, previously inactive, following a single copulation with an active male, have shown the transfer of as much as 5 per cent. of the male's activity in the form of sperm. The loss rate through excretion is very similar to that found by King (1953) following *adult* feeding.

The very rapid loss of P^{32} from adults will reduce the expected cumulative effect of radioactive decay, but it cannot eliminate it or even *reverse* it as appears from the graph of fig. 1. This must be due to the variation in sensitivity to mutagens during spermatogenesis.

6. SENSITIVITY OF DEVELOPING SPERM

The best evidence available on the variation in sensitivity is in the work of Lüning (1952) who followed the incidence of dominant lethals in consecutive sperm batches following a single dose of X-rays to the adult male. Maximum sensitivity was found in spermatids 6-7 days from maturity. Earlier stages became rapidly less sensitive (at least for recoverable mutations) until no mutations were found in germ cells irradiated eleven or more days prior to maturity. Caution should be used in applying these results to all sperm, because it is doubtful whether sperm matures at the same rate in adults as in larvæ and pupæ.

Thus, according to Kerkis (1933), 24-hour larvæ (seven days to emergence of imago) have only spermatogonia in their testes. Primary spermatocytes do not appear until larvæ are 50 hours old. Meiosis has not begun even by 96 hours (just prior to pupation, which lasts four days), though there are then large numbers of mature spermatocytes. Twenty-four hours later there are already morphologically mature spermatozoa and all stages of maturation divisions. The newly emerged male has relatively few immature stages in his testes, the bulk being mature sperm. Thus the oldest sperm in a male mating within 24 hours of emergence is less than five days from meiosis. It would appear, therefore, that either the mutation sensitive stage identified by Lüning is pre-meiotic, or sperm maturing throughout in the adult takes two to three days longer than the first sperm batches. The latter seems the likelier explanation.

Granted that there is an immature, highly radiosensitive stage in spermatogenesis, it becomes necessary to infer its timing directly

from our own data. The difference between the two curves of fig. 1 tells us that sperm which was in the hypersensitive stage during pupation was used on the 4th and 5th days' mating. The interval between the middle of pupation and the 4th to 5th sperm batches is seven to eight days. Thus, after all, the interval from hypersensitivity to maturity estimated from our own data, though estimated for sperm maturing during pupation, is the same as Lüning's.

7. ABSORPTION OF INTERNALLY GENERATED RADIATION

The prediction of absolute mutation rates would involve estimation of the roentgen equivalent dose at the testes. This is not at present possible. The mean whole-body dose, however, can be estimated from the percentage absorption of internally generated radiation.

Bateman and Sinclair (*loc. cit.*) tried to estimate this on a purely physical basis, using as a model *Drosophila* a sphere of 1 c.mm. volume. They estimated that with uniform distribution of the activity the absorption would be 38 per cent. There can now be little doubt that this was a considerable over-estimate.

King (1952) made two estimates. He used a more refined geometrical model of the fly, considering it as three spheres, head, thorax and abdomen, and made direct measurements of the distribution of activity between these three divisions. Summing the absorption to be expected on physical grounds in the three spheres, he estimated the total internal absorption as 12·55 per cent. However, so many assumptions have to be made (of which the one most likely to be erroneous is that of uniformity of distribution) that a direct measurement of absorption, however crude, is much to be preferred. King ashed male flies, which yielded a mass which was 3 per cent. of the fresh weight and obtained an increase of 9·5 per cent. in the count. Neglecting absorption by the ash, the absorption by the fresh body will be 9·5/109·5 : 8·7 per cent.

We have now made further attempts at direct measurement. Newly hatched males fed as larvæ were counted fresh and then divided into two batches, each of 10 flies. The first was squashed flat in a fold of paper which was then opened out and recounted (under these conditions there was very rapid drying). The count was increased by 9·2 per cent. The second batch was digested in sulphuric acid and counted with a liquid counter calibrated against the usual dry counter. This should have given the total activity. However, the results were very erratic (the *increase* in count varied from +1 per cent. to −13 per cent. !) and the method is evidently unsuitable. Perhaps the digestion was too incomplete. Comparison with King's results indicates that squashing the fly on paper gives as complete a count as any other method, perhaps because the fly is then in a very thin dry film. It gives us an estimate of absorption in the fresh fly of 8·4 per cent. compared with King's estimate of 8·7 per cent.

According to King, the P^{32} in the head and thorax can be ignored in estimating the radiation to the testes. The abdomen generally contains about half the total activity.

8. PREDICTION OF MUTATION RATE

Without concerning ourselves with absolute mutation rates, the relative magnitudes in different batches of sperm will be given by the sum of the products of the radiation sensitivity at each stage of maturation and the counting rates of the sources of radiation. These sources are ingested P^{32} as given in fig. 2*a*, and external radiation, which extends to the third day for flies removed from the active medium at pupation and to the seventh day for those pupating in the medium.

Counts of the surface of the food medium and pupæ show that the specific activity of the pupæ is four times that of the food. Thus if the energy released per unit time within the pupa is n ergs that released in a similar volume of food will be $0{\cdot}25\ n$.

If the absorption of internally generated radiation is 8 per cent. the resultant dose to the fly will be $0{\cdot}08\ n$.

In a continuous medium the dose to a volume of food equal to the pupa will be $0{\cdot}25\ n$, but the dose to the pupa itself will be less than this by the amount of energy generated within that volume of food which would be absorbed by it. If the absorption were 8 per cent. (as in the fly) the pupal dose from the medium would be $0{\cdot}23\ n$ or $2{\cdot}9$ times the dose from ingested P^{32}. If, at the other extreme, the absorption were 38 per cent. (the theoretical estimate for a sphere equal in volume to the pupa) the dose from the medium would be $0{\cdot}15\ n$ or $1{\cdot}9$ times that from ingested P^{32}.

Allowing for the many possible sources of error in the above calculations, the most we can confidently conclude is that, during pupation in the medium, the mutagenic potencies of external and internal P^{32} will be of the same order of magnitude.

So far no exact quantitative study has been made of the variation in sensitivity of the germ cells though Lüning concluded it was about two-fold. As hyperploid production is a two-event phenomenon its frequency will be expected to vary with the square of the sensitivity and thus exaggerate its variation. It would thus seem appropriate to concern ourselves only with the most sensitive stage and to see what variation in mutation rates would be predicted from this alone. This we will take to be seven days from maturity, or, in the experiment, the day of insemination.

Fig. 3 shows the curve of fig. 2*b* (mated males) moved seven days to the right. The Y axis is the mutation rate in arbitrary units. Superimposed on the mutations attributable to ingested P^{32} are the mutations attributable to irradiation from the medium, firstly before pupation and secondly during pupation. Comparison of figs. 1 and 3

shows a striking parallelism. One must conclude that if a prediction of mutation rates based only on the hypersensitive stage gives such a close fit, it must be much the most important mutagenically.*

The most serious discrepancy between the expectation and observation is after the ninth day, when the expected mutation rate is consistently too high. A possible explanation for this is that excessive mating has depleted the gonads of P^{32} more than the rest of the animal. As the gonadal radioactivity is the most important mutagenically, the gross activity of the fly would lead to an over-estimate of its mutagenic potentiality.

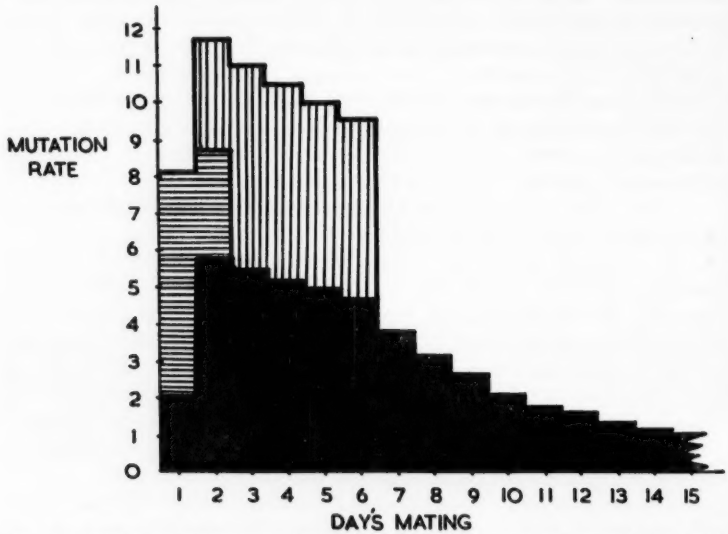


FIG. 3.—Expected mutation rates (arbitrary units) attributable to internal P^{32} (solid black), to medium external to larvæ (horizontal shading), and to medium external to pupæ (vertical shading), according to successive days' mating. *Cf.* fig. 1.

Another discrepancy is that *both* curves of fig. 1 should show a peak on the second day as seen in fig. 3. But if the first sperm batches did not reach their hypersensitive stage until after pupation (see Kerkis), prepupal radiation could be neglected. Then the curve for flies pupating out of the medium should show no peak but should start at its maximum. The existing peak is, however, of little importance as it is based on a very small sample (see table 3). The curve for flies pupating in the medium, however, should also be high from the beginning. This discrepancy cannot easily be accounted for. Otherwise, the fit between figs. 1 and 3 is good.

* More recent work by the author, using X-rays, has shown that the increased sensitivity of immature sperm is much greater for hyperploid production than for dominant lethals. In the present studies, therefore, the sensitivity of mature sperm can be ignored.

9. VALIDITY OF COMPARISONS OF P^{32} AND X-RAYS

The most obvious basis of comparison for the mutagenic actions of P^{32} and X-rays is their efficiency. But for this one must be able to express the dosages in the same units, such as roentgen. The non-random distribution of P^{32} makes it out of the question at this stage to estimate the dosage to the gamete chromosomes. Other comparisons that can be made concern the relative frequencies of the different kinds of mutation, which we may call the mutation spectrum, and the effects of the time factor.

As we have seen, the differences between the interactions of P^{32} and X-rays with time can be attributed solely to the technicalities of the treatments. The action of the P^{32} is by its very nature continuous and subject to the physiology of the organism. X-rays as normally administered are given at a point in time determined by the experimenter. The effects of P^{32} as studied above could presumably be reconstructed (laboriously) by a continuous and continually changing dose of X-rays.

The time factor is, however, of importance also to the study of the mutation spectrum, for, as is well known, the so-called " one-hit " changes increase linearly with dose, whilst " two-hit " changes increase with a higher power. Consequently, since the effective dose from the two mutagens changes differently with time, the mutation spectrum will change also. One must therefore restrict comparisons to a well-defined sperm batch, with dosages of P^{32} and X-rays adjusted to give the same rate for one " band " of the mutation spectrum (say sex-linked lethals).

A further complication has come to light, details of which I shall be publishing shortly. The variation in sensitivity during sperm maturation to one and the same mutagen (X-rays) is markedly different according to the " band " being studied. It is not sufficient therefore to confine one's attention to a single sperm batch. The two mutagens must have acted on the *same* maturation stage. With P^{32} and X-rays this would be almost impossible to achieve.

It appears, then, that one can have no confidence in attributing any difference which has been or may in future be revealed between the effects of these two mutagens to the quality of the radiations (such as the transmutation of P^{32} to S^{32}). There are too many other possible sources of difference.

10. SUMMARY

1. Preliminary comparisons of the mutagenic effects of ingested P^{32} and X-radiation of mature sperm showed the need to analyse the variation in the effects of P^{32} with successive sperm batches.

2. *Drosophila* males were reared in vials of normal medium to which had been added 30 μC of P^{32} in 0·03 ml. of solution twenty-four hours after termination of egg laying.

3. The mutation rate was measured as the percentage of hyperploid non-*yellow* daughters on mating irradiated males to fresh attached-*X yellow* females each day.

4. In males pupating out of the active medium the mutation rate was about constant at 3 per cent. up to the sixth day's mating, when it fell to zero by the ninth day. Males which had pupated in active medium showed an additional peak of 7 per cent. to 8 per cent. on the fourth and fifth days' mating.

5. The P^{32} content of the males was maximal during pupation. Adults lost their activity rapidly, especially when mated.

6. It was confirmed that there is a hypersensitive stage in spermiogenesis seven days prior to maturity.

7. The absorption of internally generated radioactivity was reassessed at 8 per cent.

8. The variation in mutation rate with day of mating was predictable from the P^{32} content of the fly and the extent of external radiation at the moment when each batch was in its hypersensitive stage.

9. During pupation in active medium external and internal radiation were responsible for about equal amounts of mutation.

10. The difficulties in the way of a valid comparison of the qualitative effects of P^{32} and X-rays are almost insuperable.

Acknowledgments.—The author wishes to express his appreciation to Dr F. C. Bawden of the Plant Pathology Department, Rothamsted, for permission to use its Geiger counter and to Mr H. L. Nixon of the same Department for assistance and advice with the more physical aspects of the work.

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CHROMOSOME BALANCE AND ENDOSPERM FAILURE IN HYACINTHS

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1. INTRODUCTION

FAILURE of endosperm resulting in failure of an otherwise normal embryo is a widespread occurrence following crosses between species, between plants of different chromosome number and on selfing normal outbreeders. Various suggestions have been made as to the cause of the breakdown (see Brink and Cooper, 1947 ; Beaudry, 1951 ; Brock, 1954). These suggestions mainly concern chromosomal, genic or cytoplasmic unbalances in the endosperm, or between endosperm, embryo and maternal tissue or between endosperm and antipodals.

The mechanism of endosperm failure in *Lilium* hybrids has been shown to be the accumulation of mitotic abnormalities, resulting from spontaneous chromosome breakage. These reduce the mitotic activity and culminate in degeneration of the endosperm and failure of the embryo (Brock, 1954).

Hyacinthus orientalis was selected for further work on this problem because of its triploid endosperm, its suitability for cytological examination and the unusual situation of chromosome balance operating in this species (Darlington and Mather, 1944 ; Darlington *et al.*, 1951).

As a result of an internal chromosome balance, viable gametes with different chromosome numbers are produced by aneuploid and triploid hyacinth varieties. Thus endosperms can be produced which differ in chromosome number. Endosperm failure can thus be studied in relation to both genetic and chromosomal variables.

2. METHODS

Hyacinth varieties were selfed and cross pollinated without prior emasculation. No ovules developed after selfing. Ovules from Lady Derby crossed Duke of York and the reciprocal cross were fixed at 5-day intervals for 30 days. In all other crosses the ovules were fixed between 12 and 16 days after pollination. At each fixation and when harvesting the mature seeds, the total ovules and the numbers of developing and degenerating ovules were recorded.

Fixations 5 days after pollination were made in Navashin's fluid (Darlington and La Cour, 1947). These were embedded in wax, section cut 16 μ thick and stained in crystal violet. All other fixations were made in Carnoy's fluid (6 : 3 : 1). After storage in absolute alcohol, 1 to 14 days, ovules were hydrolysed for 13 minutes in N/1 HCl and stained in Feulgen reagent. The embryo and endosperm were dissected and squashed in 45 per cent. acetic acid. Slides were made permanent by mounting in Euparal.

In the X-ray experiments pollen and ovules were irradiated immediately before pollination, developing endosperms 9, 12 and 13 days after pollination. All

fixations were made 14 days after pollination. The irradiation was unfiltered at 70 Kv. and 5 m.a., focal distance 20 cm., dosage rate 51·5 Röntgen units per minute. Total dose for pollen and unfertilised ovules was 500 r and for endosperms 100 r.

3. CHROMOSOMES OF THE VARIETIES

One of the reasons for choosing hyacinth for this work was the variation in its chromosome complement. Darlington *et al.* (1951) have described the chromosomes of 106 varieties. For convenience the chromosome complement of the varieties now used is given in table 1.

TABLE 1

Somatic chromosome complements of hyacinth varieties

	Chromosome complement				
	L	L^n	M	S_1	S_2
2*x*—					
Borah, Cherry Blossom, Early Roman, *H. orientalis*	6	2	4	2	2
	Extra chromosomes				
3*x*—					
King of the Blues, Lady Derby	3	1	2	1	1
Hypo-3*x*—					
Rosalie (2*x*+1)	...	1	...	...	...
Duke of York (2*x*+6)	3	1	...	1	1

Note.—The chromosome complement of Duke of York used here has seven chromosomes less than that given by Darlington *et al.* (1951). My plants were obtained from a different source and probably represent a misnomer due to the mauve flower colour of the two clones.

The haploid set of eight consists of four long chromosomes, (L), one of which carries the nucleolar organiser (L^n); two medium chromosomes (M); and two short chromosomes which can be separated on the position of the centromere into S_1 and S_2.

Five plants of *H. orientalis* collected in Palestine by Major A. E. Pam were used. Two were normal diploids, the other three each had a deficiency of the whole of the long arm of one of the S_2 chromosomes.

Nucleolar constriction. In the rapidly dividing endosperm the chromosomes at metaphase are incompletely spiralised and they are in general 20 to 25 per cent. longer than root tip chromosomes. The nucleolar constriction is much attenuated and two types of L^n chromosomes are recognisable in the endosperm. In one type the nucleolar

constriction is very long and appears as a weakly staining gap in the chromosome. In the other the nucleolar constriction is shorter and has three deeply staining knobs in it (pl. 1, fig. 2). These two types of L^n chromosomes can occasionally be recognised in root tip preparations (Darlington *et al.*, 1951) but here, owing to the greater spiralisation, only slight differences in the length of the constriction are observed. From the crosses that have been examined it appears that Borah, King of the Blues, Lady Derby and Rosalie are heterozygous for this condition ; Early Roman and *H. orientalis* had only the type with the shorter constriction with staining areas and Cherry Blossom only long constrictions.

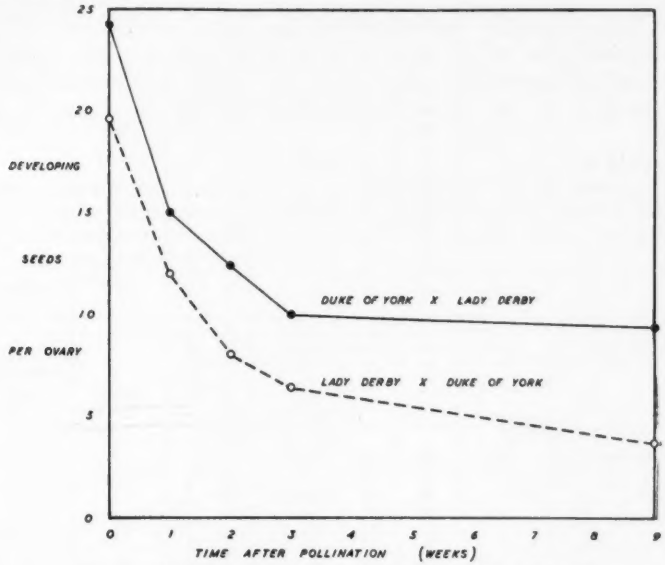

FIG. 1.—Graph showing failure of developing seeds in reciprocal hyacinth crosses at various times after pollination.

The staining areas in the attenuated constriction region probably represent small structural changes in the chromosomes.

4. CROSSING AND SEED SET

No ovules developed or seeds set after self pollination but many crosses between the varieties were successful. The seed set per ovary was low in all crosses. Examinations at intervals after pollination showed that the main period of failure of the developing seeds was during the first three weeks after pollination (fig. 1). This was due to the failure of the endosperm and subsequent abortion of the embryo and is comparable to the endosperm failure previously reported in *Lilium*. As in *Lilium* no mitotic abnormalities occurred in the hyacinth

embryos. The mitotic abnormalities in the hyacinth endosperms were not, however, so severe as those in *Lilium*. This may have been due in part to the triploidy of the hyacinth endosperm as opposed

TABLE 2

Hyacinth variety crosses and chromosome complements of resulting endosperms 12 to 16 days after pollination

Cross ♀ by ♂	No. of endosperms	Chromosome complement					Mean chromosome no.	
		3*n*	L	L[a]	M	S	Expected	Observed
2*x* by 2*x* .	8	24	9	3	6	6	*24·0*	24·0
2*x* by 2*x*+1 .	1	24	9	3	6	6	*24·5*	24·0
2*x*+1 by 2*x* .	16	24	9	3	6	6		
	6	26	9	5	6	6	*25·0*	24·5
2*x*+1 by 3*x* .	1	31	12	5	6	8		
	1	31	12	3	9	7		
	1	29	12	3	7	7		
	2	29	11	4	7	7		
	1	28	12	4	6?	6		
	1	27	12	3	6	6		
	1	27	11	3	7	6		
	1	27	11	3	6	7	*29·0*	28·7
2*x*+6 by 2*x* .	1	32	13	5	6	8		
	1	30	13	5	6	6		
	1	28	13	3	6	6		
	1	26	11	3	6	6	*30·0*	29·0
3*x* by 2*x* .	3	38	15	5	8	10		
	1	36	15	3	8	10		
	1	34	13	5	8	8		
	1	34	11	5	10	8?		
	1	32	13	3	8	8		
	1	32	11	5	10?	6		
	1	32	11	5	8	8		
	1	32	11	5	6	10		
	1	30	13	3	8	6		
	1	30	11	5	8	6		
	1	30	11	5	6	8		
	1	28	9	5	8	6		
	1	26	11	3	6	6		
	1	26	9	3	6	8	*32·0*	32·2
3*x* by 2*x*+1 .	1	37	13	6	8	10		
	1	32	15	3	8	6		
	1	32	13	3	6	10		
	1	32	9	5	10	8		
	1	26	11	3	6	6	*32·5*	31·8

to its pentaploidy in *Lilium*. On the other hand the hyacinth's aneuploids are almost as satisfactory as balanced multiples of the chromosome set: the hyacinth can afford these errors because of its unusual chromosome balance.

5. CHROMOSOME BALANCE

The distribution of different types of chromosomes has been studied by Darlington and Mather (1944) in pollen grains of triploid hyacinths and by Darlington *et al.* (1951) in aneuploid commercial hyacinth varieties. The pollen grains were virtually unselected and demonstrate the balance between the chromosomes. The horticultural varieties, which have been selected both for vigour and flower characters, indicate not only the breeder's preference but also the chromosome complements which are favoured as a permanent basis of growth.

From studies on pollen grains of triploid hyacinths the total loss of chromosomes at meiosis has been placed at 10 per cent. by Belling (1924), Darlington (1926) and Darlington and Mather (1944) and at 5 per cent. by Darlington *et al.* (1951). These last two papers also establish the principle that there is an increasing frequency of loss at meiosis with decreasing chromosome size.

TABLE 3

Contribution of extra chromosomes in gametes which were successful in crosses (table 2). The complements of the gametes calculated from the endosperm complement (table 2) and the known complement of one gamete (x or $x+L^n$). Calculation based on monosporic embryo sac development (Anderson, 1936)

Parent	♀			♂		
	No. of gametes	Extra chromosomes		No. of gametes	Extra chromosomes	
		Mean per gamete	Loss or gain		Mean per gamete	Loss or gain
$2x+1$	31	**0·23**	$-0·27$	6	**0·17**	$-0·33$
$2x+6$	4	**2·5**	$-0·50$	...	...	...
$3x$	21	**4·0**	0	9	**4·3**	$+0·3$

Darlington *et al.* (1951) examined the chromosome balance in somatic cells of commercial hyacinth varieties. They found a correlation between L and M+S chromosomes which depended on the absence of certain unbalanced types (4 : 2 and 2 : 4). There were correlations between the numbers of extra M and S chromosomes and between the extra L and L^n chromosomes. There was also a preference for odd numbers of L and S chromosomes and even numbers of M chromosomes.

Developing seeds represent an intermediate step in the selection processes. Chromosome complements have been determined in the endosperm, and in some cases in the embryo also, of hybrids 12 to 16 days after pollination (table 2). At this time the embryo consisted

of 16 to 25 cells and the endosperm 250 to 500 free nuclei in a common cytoplasm. Mitoses in the endosperm were synchronous.

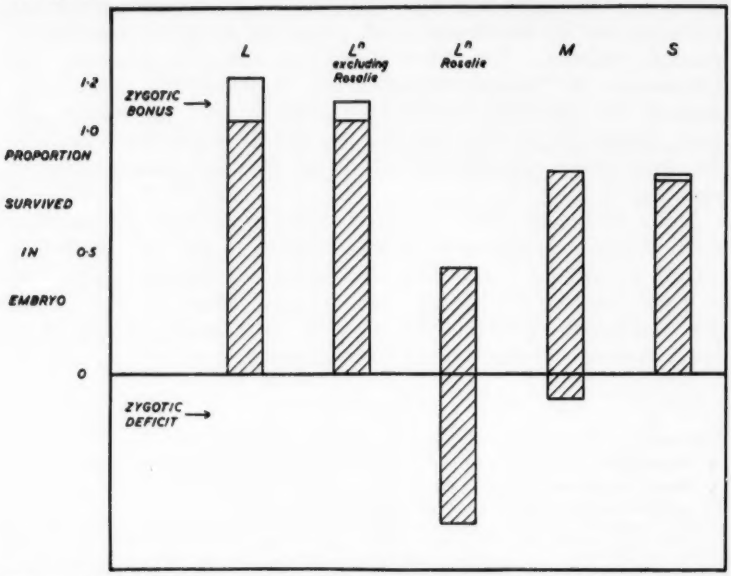

FIG. 2.—Survival of chromosomes of each type in pollen grains (hatched), from Darlington *et al.* (1951), compared with survival in embryo 12 to 16 days after various crosses (table 2). Survival of L and Lⁿ not shown separately by Darlington *et al.* (1951), their combined figure for L+Lⁿ is compared with both L and Lⁿ in embryos. Zygotic bonus (clear) represents increased survival and zygotic deficit decreased survival in embryos.

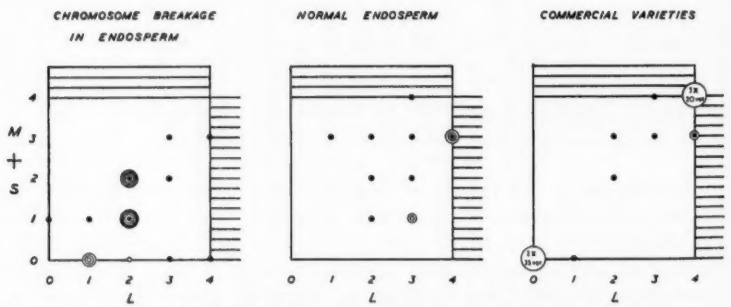

FIG. 3.—Relation of L to M+S in the extra chromosomes of hyacinths. Hybrid embryos, 12 to 16 days after pollination, from the various crosses (table 2) compared with commercial varieties (Darlington *et al.*, 1951). Each circle represents one variety or hybrid. The four crosses involving Duke of York ($2x+6$) indicated by open circles. Rosalie by diploid crosses not included (see table 5). $L = (L+L^n)$.

Knowing the chromosome complements of the endosperm and one gamete, the complements of the other gamete and the embryo can be calculated.

TABLE 4

Number of extra chromosomes of each type, derived from ♀ and ♂ parents surviving in embryos 12-16 days after various crosses (table 2)

Type	Derived from	2n+	No. of embryos	Extra chrs.	Mean exp'd.	Mean obs'd.	Prop'n. survived	
L . . .	♀	0	3					
		1	9					
		2	8					
		3	5					
			25	40	1·50	1·60	1·07	1·22
	♂	2	5					
		3	4					
			9	22	1·50	2·44	1·63	
L^n . . Excluding Rosalie	♀	0	10					
		1	15					
			25	15	0·5	0·6	1·20	1·12
	♂	0	5					
		1	4					
			9	4	0·5	0·44	0·88	
L^n . . Rosalie	♀	0	24					
		1	7					
			31	7	0·5	0·23	0·46	0·43
	♂	0	5					
		1	1					
			6	1	0·5	0·17	0·34	
M . .	♀	0	6					
		1	12					
		2	3					
			21	18	1·0	0·86	0·86	0·83
	♂	0	4					
		1	4					
		2	0					
		3	1					
			9	7	1·0	0·78	0·78	
S . . .	♀	0	10					
		1	8					
		2	7					
			25	22	1·0	0·88	0·88	0·82
	♂	0	3					
		1	6					
			9	6	1·0	0·67	0·67	

L^n excluding Rosalie : L^n Rosalie. $\chi^2 = 11{\cdot}4$; $P < 0{\cdot}01$.

The successful gametes from the triploid parents had a mean of four extra chromosomes per gamete (table 3). This suggests no loss of chromosomes at meiosis and is contrary to the previous accounts (Belling, 1924; Darlington, 1926; Darlington and Mather, 1944; and Darlington *et al.*). Examination of the survival of the individual types of chromosomes in the embryo reveals that there was, in fact,

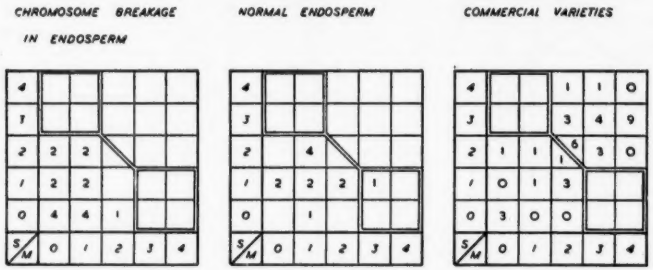

FIG. 4.—Relation of M to S in the extra chromosomes of hyacinths. Comparison of developing seeds and commercial varieties. The same varieties and hybrids as in fig. 3 except that the Duke of York ($2x+6$) crosses are excluded.

loss at meiosis. This loss agreeing with the principle that in polysomic plants there is an increasing frequency of loss at meiosis with decreasing chromosome size (table 4, fig. 2). These embryos therefore represent selective fertilisation by the gametes with higher chromosome number or selective survival of embryos resulting from fertilisation with such gametes.

TABLE 5

Relation of odd to even numbers in each type of chromosome in embryo 12-16 days after pollination

	Chromosomes in embryo							
	L		M		S		M+S	
	Odd	Even	Odd	Even	Odd	Even	Odd	Even
No breakage in endosperm .	7	7	8	4	8	6	10	4
Breakage in endosperm .	7	12	8	9	4	15	8	11
Total . . .	14	19	16	13	12	21	18	15
Commercial varieties preference for	Odd		Even		Odd		Odd?	

The chromosome complements of the embryos confirm the number relations of L to M+S chromosomes evident in the established varieties (fig. 3). However, the preference for the odd numbers of L and S

chromosomes and even numbers of M chromosomes observed in the commercial varieties is not confirmed in either the total embryos or those with endosperms which showed no chromosome breakage (table 5, fig. 4). Also there are exceptions to the selective concentrations of extra M and S chromosomes and the number relations of L to L^n chromosomes outlined by Darlington *et al.* (1951). Perhaps these represent chromosome combinations which, although surviving the selective mechanism of endosperm failure, will be eliminated by later selection processes.

One notable exception results from an unusual male gamete from the triploid King of the Blues. This gamete had a complement 6 : 1 : 5 : 3, and was formed by 5 of the 6 M chromosomes segregating into the one pollen grain. This could only occur by illegitimate

TABLE 6

Number of abnormal endosperms in various hyacinth crosses, 12-16 days after pollination

Cross ♀ by ♂	Mean extra chrs.	Number of endosperms				
		Total	Degenerating	Dividing	Sub-chrd. and chr. breakage	Spindle errors
2x by 2x .	0	283	3	17	6	2
2x by 2x+1	0·5	501	6	39	14	6
2x+1 by 2x	1·0					
2x by 3x .	4·0	436	13	26	11	5
2x+1 by 3x	5·0					
3x by 2x .	8·0	906	41	65	40	18
3x by 2x+1	8·5					
2x+6 by 3x	10·0	758	36	59	38	13
3x by 2x+6	11·0					
		2884	99	206	109	44

segregation of a univalent or by quadrivalent formation (which has not been reported in triploid hyacinths).

Early degeneration of the developing seeds and chromosome breakage in the endosperm both increase in frequency as the chromosome number of the parent varieties is increased (table 6, fig. 5). Although there is this relationship between chromosome number and failure of the endosperm it is also apparent that abnormalities occur where there are no extra chromosomes (diploid by diploid crosses).

Rosalie. The extra L^n chromosome in Rosalie was less readily transmitted to the embryo and endosperm than the extra chromosomes of other varieties (tables 2, 3, 4).

At first metaphase of meiosis in Rosalie, chiasma frequency is high, mean chiasma frequency per potential bivalent in 10 cells was 2·84, and the L^n chromosomes formed trivalents in 40 of 47 cells. A high frequency of bridges and fragments, resulting from crossing

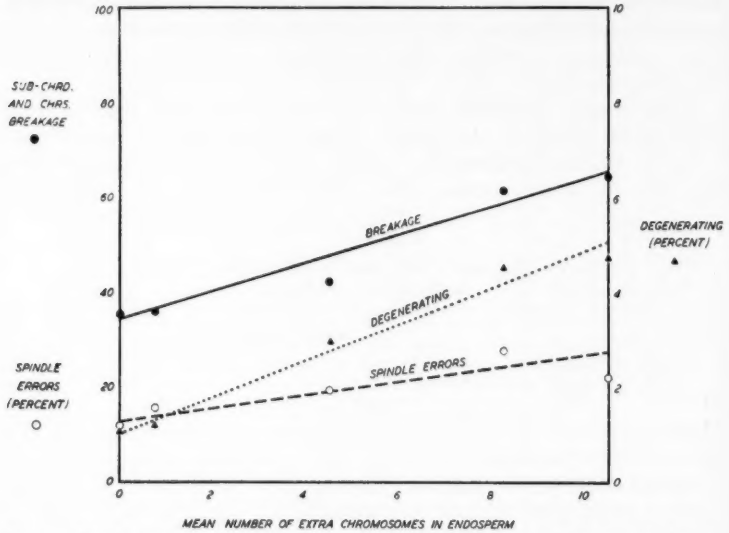

FIG. 5.—Graph showing relation of extra chromosomes in hyacinth endosperms to chromosome breakage, degeneration and spindle abnormalities. Various crosses (table 2), 12 to 16 days after pollination.

over in inverted segments, occurred at A I and A II (table 7). These always involved L and never L^n chromosomes. Several first anaphases had two separate bridges and fragments and one mother cell at A II had bridges and fragments in each of the two daughter cells. Short

TABLE 7

Inversion crossing over (I.C.O.) frequency at meiosis in the variety Rosalie

A I		A II		I.C.O. frequency
Mother cells	b+f	Mother cells	b+f	
235	98	155	38	0·66

Note.—I.C.O. frequency calculated by adding together the frequencies of bridges at first and second division per mother cell (Darlington, 1937, p. 271).

fragments were most frequent but occasionally a long fragment accompanied by a short bridge occurred. Thus at least two pairs of L chromosomes had relatively inverted segments.

At second division of meiosis daughter cells were always synchronised and there was no evidence of any loss of L^n chromosomes.

Counts at pollen grain mitosis showed only 16·5 per cent. of cells with the extra L^n chromosome and less than 3 per cent. of cells with evidence of chromosome breakage (table 8). At this stage approximately 30 per cent. of the pollen grains were degenerating and appeared

TABLE 8

Numbers of pollen grains of Rosalie (trisomic for L^n) with 1 or 2 L^n chromosomes

	1 L^n	2 L^n
Normal	94	18
One L with SR . . .	1	1
Two L's as dicentric . .	1	...
	83·5 per cent.	16·5 per cent.

incapable of further mitosis. All of the anthers examined were at an early phase, less than 25 per cent. of the normal-looking pollen grains were binucleate. Later phases may show a higher frequency of pollen grains with the extra L^n chromosome but Darlington and Mather (1944) found that in a triploid hyacinth extra chromosomes had no effect on the rate of entry into pollen grain mitosis.

TABLE 9

Effect of extra L^n chromosome from Rosalie on endosperm 12-16 days after pollination

Endosperm complement	Total endosperms	Endosperms with breakage	Total anaphases	Anaphases with breakage	Per cent.
$3n$	20	8	791	44	*5·56*
$3n+L^n$	1	1	33	15	*45·45*
$3n+2L^n$	5	4	334	20	*5·99*

There is no evidence relating the structural hybridity in the L chromosome with the apparent loss of extra L^n chromosomes in the pollen grains. This loss could account for all, or part of, the shortage of L^n chromosomes in the developing endosperms and embryos in Rosalie crosses (table 4). Some of this shortage may, however, result from early degeneration of the ovule.

There is evidence that additional L and L^n chromosomes, when accompanied by additional M and S chromosomes, benefit the

developing seeds (fig. 2). But the endosperms where the extra chromosomes are all of the one type nearly always have chromosome breakage (figs. 3 and 4). Thus it appears that the addition of only one type of chromosome upsets the balance and results in chromosome breakage and early degeneration.

There is some evidence of this in the endosperms resulting from 2*x* by Rosalie crosses (table 9). This may appear contrary to the fact that the extra L^n chromosome in Rosalie protects against observed X-ray breakage (La Cour, 1952). These data are not necessarily contradictory, for the protection against observed X-ray breakage is associated with a changed physiological condition and acts presumably by favouring restitution. This altered physiology may, when acting in the special endosperm tissue, favour greater spontaneous chromosome breakage.

6. THE MECHANISM OF ENDOSPERM FAILURE

(i) *Sub-chromatid errors*

The first cytological abnormalities are errors in anaphase separation (plate I, fig. 1). La Cour and Rutishauser (1953*a*) describe similar errors in *Scilla* endosperms after X-raying and consider that they result from sub-chromatid breakage and reunion. Earlier accounts have termed these errors point stickiness or " pseudo-chiasmata ". Similar errors have arisen at meiosis after genotypic changes : mutations in maize (Beadle, 1932), segregation by inbreeding in rye (Lamm, 1936 ; Müntzing and Akdik, 1946) and hybridity in a species cross in *Chorthippus* (Klingstedt, 1939). They are induced by environmental changes and are characteristic of the " primary " effects of X-rays (Darlington and La Cour, 1945). They also occur at mitosis and meiosis after chemical treatment with mustard gas (Darlington and Koller, 1947), phenols (Levan and Tjio, 1948) and coumarin (Östergren, 1948).

In these hyacinth endosperms the errors were either intercalary or terminal and in one case involved non-homologous chromatids (L and L^n). There were more terminal than intercalary associations and, as has been suggested by Darlington and Upcott (1941), some of these could result from failure of reproduction of the end genes.

Anaphase separation after sub-chromatid breakage and reunion results in either breakage of the chromatids or breakage of the association. When the association breaks it results in anaphase chromatids which are much attenuated between the centromere and the point of attachment and probably also accounts for the non-nucleolar constrictions or half-breaks occasionally observed at metaphase. In these endosperms the chromatids pulled apart more frequently than they broke. However, sub-chromatid associations formed after X-raying more frequently resulted in chromatid breakage (La Cour, by personal communication).

(ii) *Chromosome breakage*

Where anaphase separation results in mechanical breakage of the chromatids the broken ends are successively available for chromosome

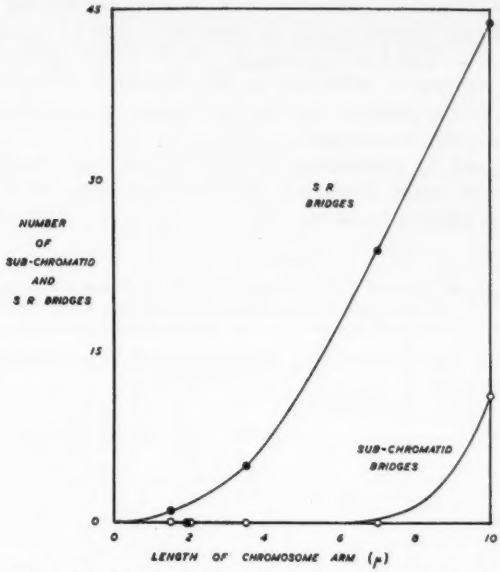

FIG. 6.—Relation of the length of chromosome arm with the number of sub-chromatid and SR bridges occurring in endosperm, various crosses (see table 2), 12 to 16 days after pollination.

TABLE 10

Comparison of sub-chromatid association and SR bridges in L, M and S chromosomes in endosperms, 12 to 16 days after pollination. Correction for length and frequency will give 1 : 1 : 1 ratio if distribution random. Chromosome lengths L = 19 μ. M = 9 μ. S = 5 μ. ($L = L + L^n$)

Ratio of L : M : S

	(2x) × (3x)	(2x+6) × (2x)	(2x+6) × (3x)	(3x) × (2x+6)
Chromosome no. .	2 : 1 : 1	16 : 6 : 8	18 : 7 : 9	18 : 8 : 9
Sub-chromatid B+R	33 : 0 : 0	...	3 : 0 : 0	9 : 0 : 0
SR bridges . .	152 : 23 : 5	7 : 1 : 0	11 : 0 : 0	6 : 0 : 1
Corrected for length and frequency				
Sub-chromatid B+R	4·3 : 0 : 0	...	0·4 : 0 : 0	1·2 : 0 : 0
SR bridges . .	20·0 : 12·8 : 5	0·9 : 0·7 : 0	1·5 : 0 : 0	0·8 : 0 : 1

reunion (R″), sister reunion (SR) or chromatid reunion (R′) (La Cour, 1952). As breakage of a single anaphase bridge is the most frequent occurrence, SR is the most frequent consequence. This

initiates the bridge-breakage-fusion cycle in the endosperm inferred by McClintock in maize (1941) and recently demonstrated in *Lilium*. The number of SR bridges was related to the length of the chromosomes (the longer the chromosome the more frequent the SR bridges) and sub-chromatid breakage only occurred in L and L^n chromosomes (table 10, fig. 6). This is in agreement with the results of Rees (1952), who showed centromere influence on the formation of subchromatid associations. It also suggests that the SR bridges are largely initiated by breakage of previous anaphase associations.

In the L and L^n chromosomes both the SR and sub-chromatid bridges occurred more frequently in nucleolar arms than in non-nucleolar arms (table 11 and fig. 7).

TABLE 11

Comparison of sub-chromatid and SR bridges in arms of L and L^n chromosomes in endosperms, 12-16 days after pollination

	Endosperms	Anaphases	Ratio of chrs. arms in endosperm		Number of anaphase bridges	
			Nucleolar L^n	Non-nucleolar L^n L	Nucleolar L^n	Non-nucleolar L^n L
Sub-chrd. associations	6	29	1	1 7·3 (8·3)	8	3 18 (21)
Nucleolar : Non-nucleolar $\chi^2 = 8{\cdot}67$ $P<0{\cdot}01$						
SR bridges	27	106	1	1 5·7 (6·7)	17	3 26 (29)
Nucleolar : Non-nucleolar $\chi^2 = 23{\cdot}66$ $P<0{\cdot}01$						

Note.—2, 4, 8, etc., nuclei occurring together each with a bridge in the same chromosome arm were scored as one bridge.

McLeish (1952) has demonstrated a similar preference for SR in the nucleolar arm of *Vicia faba* after chromosome breakage induced by maleic hydrazide. It is interesting that chemically induced chromosome breakage and spontaneous sub-chromatid breakage both follow the same pattern in this respect and suggests that both may be due to an indirect effect on chromosome reproduction with which the nucleolus is intimately concerned. The prevalence of abnormalities in the nucleolar arm is further confirmed by the high proportion of rings and dicentrics which involve this chromosome (table 12). Rings and dicentrics, once formed, persist either by parallel separation or through the bridge-breakage-fusion cycle. It is notable here as in the *Lilium* endosperm that rings, in addition to persisting, tend to

accumulate. As many as 7 rings were observed in the one anaphase, suggesting that special conditions of breakage or reunion were necessary for ring formation.

TABLE 12

Proportion of L, M and S chromosomes in endosperms 12-16 days after pollination, involved in cytological abnormalities other than sub-chromatid or SR bridges. All crosses from table 2. (Ratio L : L^n : M : S approximately 3 : 1 : 2 : 2)

	Total	L	L^n	M	S
centrics * . . .	38	23	6	5	4
ngs	6	2	4	...	...
lophase laggards . .	7	1	6	...	...
eaks at centromere .	4	2	2	...	...
	55	28	18	5	4

* 19 dicentrics, 38 centromeres from 38 chromosomes. All M and S are coupled with L or L^n.

Lagging telophase chromatids (*cf.* Dowrick, 1953 ; Haque, 1952 ; Kihlman, 1952 ; Mota, 1952) and breakage at the centromere disproportionately often, involved the L^n chromosome.

Although breakage did accumulate, the " snowballing " effect of breakage inducing more breakage, which culminates in degeneration, was less apparent in the hyacinth endosperm than in the lily.

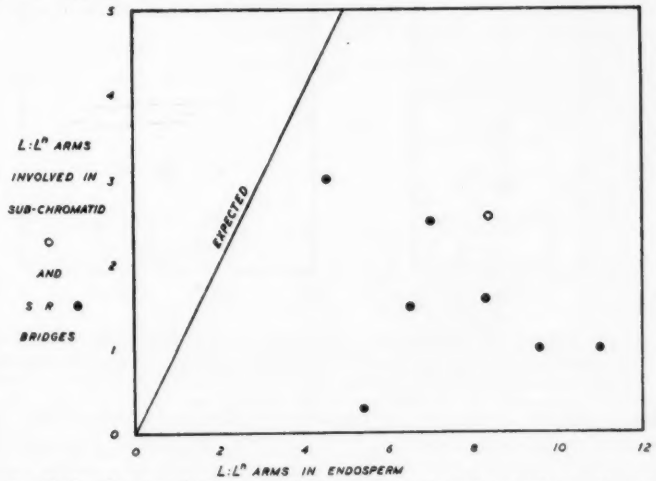

FIG. 7.—Ratio of non-nucleolar to nucleolar arms involved in sub-chromatid and SR bridges in endosperms. Various crosses (see table 2), 12 to 16 days after pollination.

Although breakage is the result of unbalance (chromosomal, genic or cytoplasmic) the chromosome complement of the hyacinth is no doubt buffered against the effects of this breakage and further abnormalities accumulate only slowly. This suggests that the balance

of the hyacinth chromosomes may extend, beyond individual chromosomes, to internal balance of broken or deficient chromosomes. Two observations support this suggestion. First, healing against reunion occurs. Although the bridge-breakage-fusion cycle operates in these endosperms, healing against reunion occasionally results in failure of bridge formation and conversion of rings and dicentrics into ordinary chromosomes. It was not possible to estimate the frequency of this

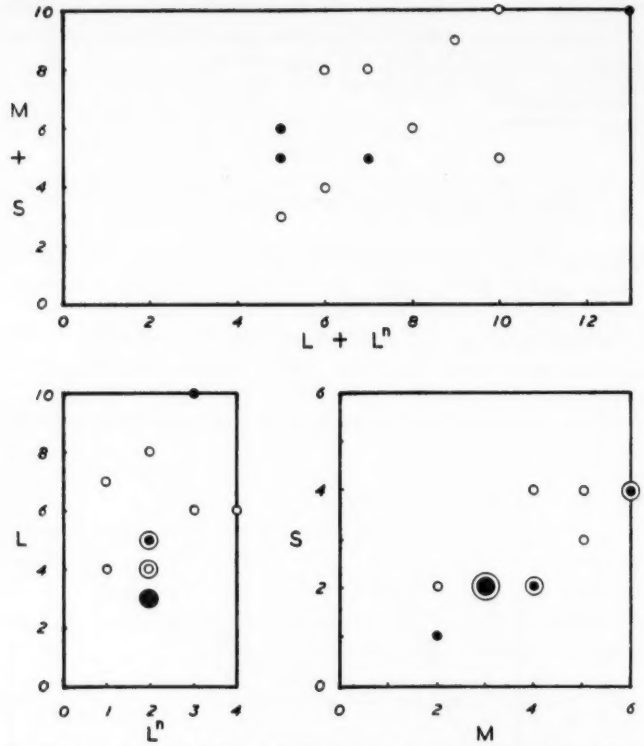

FIG. 8.—Relation between L, M and S chromosomes in aneuploid nuclei resulting from split spindles, occurring in endosperms 12 to 16 days after pollination. Each group of aneuploid nuclei represented by circle. Solid circles indicate chromosome breakage.

healing against reunion. Secondly, a deficient chromosome was transmitted through the pollen. Where the diploid *H. orientalis* with a telocentric and deficient S2 chromosome was used as a pollen parent the deficient chromosome was transmitted by the pollen to the embryo and endosperm in four of the eight crosses examined.

(iii) *Spindle abnormalities*

A general spindle abnormality is indicated by the lack of wall formation during the free-nucleate stage of endosperm development,

but in addition fusion (plate II, fig. 1) and splitting (plate II, fig. 2) of spindles occurred. Fusion of adjacent spindles is one of the mechanisms for the formation of the highly polyploid nuclei commonly observed in endosperm. Evidence that this occurs is given by such nuclei occurring in the absence of chromosome breakage and by the presence of composite endosperms : *e.g.* hexaploid nuclei with a single dicentric resulting from the fusion of a normal triploid nucleus and one containing a dicentric. The three types all occurring in the one endosperm (plate II, fig. 5).

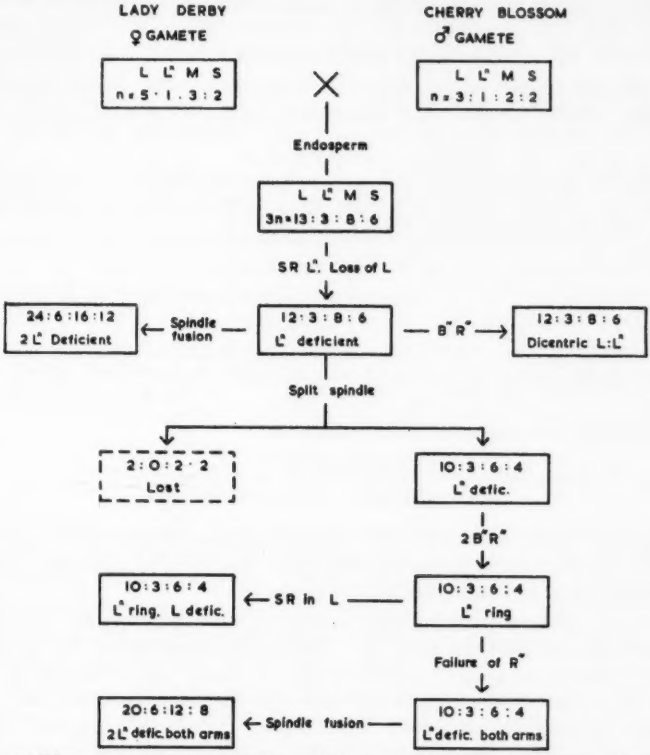
LADY DERBY
♀ GAMETE
L L' M S
n = 5 : 1 : 3 : 2
CHERRY BLOSSOM
♂ GAMETE
L L' M S
n = 3 : 1 : 2 : 2
Endosperm
L L' M S
3n = 13 : 3 : 8 : 6
SR L'. Loss of L
24 : 6 : 16 : 12
2 L' Deficient
Spindle fusion
12 : 3 : 8 : 6
L' deficient
B" R"
12 : 3 : 8 : 6
Dicentric L:L'
Split spindle
2 : 0 : 2 : 2
Lost
10 : 3 : 6 : 4
L' defic.
2 B" R"
10 : 3 : 6 : 4
L' ring, L defic.
SR in L
10 : 3 : 6 : 4
L' ring
Failure of R"
20 : 6 : 12 : 8
2 L' defic. both arms
Spindle fusion
10 : 3 : 6 : 4
L' defic. both arms

FIG. 9.—Diagram representing changes which occurred in one endosperm. Fixation 15 days after pollination. Changes inferred from complements represented in endosperm. *Note* L^n deficient means L^n chromosome which has lost a portion of one arm. It does not mean that the complement is deficient in one L^n chromosome.

Highly polyploid nuclei can also be formed by anaphase bridges failing to break. The interphase nuclei remain connected and at the subsequent metaphase, if mitoses are synchronised, restitution occurs (plate II, fig. 3).

In the aneuploid nuclei resulting from split spindles the number relationships between the L, L^n, M and S chromosomes previously demonstrated were also apparent (fig. 8). Even though these nuclei

were side by side with the normal nuclei and in a common cytoplasm the highly unbalanced types failed to persist. No aneuploid nucleus was completely deficient in any one type of chromosome and the lowest total number was equivalent to the haploid number.

The frequency of spindle abnormalities increased with increasing chromosome number of the endosperm but, as with sub-chromatid and chromosome breakage, the endosperms resulting from diploid by diploid crosses were not free from irregularities (fig. 5).

The development of combined abnormalities in an endosperm from a triploid by diploid cross is illustrated in fig. 9.

7. AGED POLLEN

All the crosses so far discussed were made with freshly dehisced pollen. Some crosses were attempted with pollen which had been

TABLE 13

Random distribution of X-ray breakage in an endosperm with chromosome complement L 12, L^n 4, M 6, S 6 (Rosalie × King of the Blues). Dose 100 r, 13 days after pollination. Fixation 24 hours after irradiation

	L	L^n		M	S	Total nuclei
		Non-nuc.	Nuc.			
SR bridges with fragments .	30	5	9	5	1	44

Nucleolar : Non-nucleolar arms of L chromosomes

$\chi^2 = 2{\cdot}55$ $P = 0{\cdot}1$ N-S

L : M : S using length (19 : 9 : 5)/frequency (32 : 12 : 12) ratio

$\chi^2 = 3{\cdot}28$ $P = 0{\cdot}2$ N-S

stored at 6° C. over $CaCl_2$ for periods varying from 16 to 34 days. The seed set following such pollinations was greatly reduced. Some developing ovules were, however, obtained.

The endosperms from pollen stored for 16 to 18 days had no more abnormalities than the controls. But from pollen stored for 34 days two endosperms showed abnormalities of kinds not observed in the control. One had chromosome and spindle abnormalities, and also single lagging L^n anaphase chromatids, in adjacent nuclei. The other, examined 16 days after pollinatior still had an unfertilised egg cell. What appeared to be a sperm n leus was lying beside the haploid egg nucleus. The endosperm cons ed of one highly polyploid nucleus which was showing mass chromosome breakage (plate II, fig. 6).

8. X-RAY EXPERIMENT

X-ray-induced chromosome breakage hyacinth endosperms was examined to see whether its distribı was random or concentrated in particular chromosomes and like the spontaneous breakage.

An endosperm fixed 24 hours after irradiation was scored for anaphase bridges with accompanying fragments (table 13). There was no significant concentration of breakage in either the long chromosomes or the nucleolar arms. The method of scoring seems to be valid but it is open to criticism on the grounds that no suitable controls were available because of the high level of spontaneous breakage in these hyacinth endosperm (fig. 5). Restricting the analysis to cells which showed bridges and fragments at the first division after treatment excludes most of the persisting bridges, which result from earlier spontaneous breakage, but it includes those which still have fragments. This would account for the slight but not significant concentration of bridges involving long chromosomes and nucleolar arms (table 13).

Other endosperms fixed 48 and 120 hours after irradiation showed all the mitotic and spindle abnormalities observed after spontaneous chromosome breakage; sub-chromatid breakage, SR bridges, dicentrics, rings, reproduced fragments, micronuclei, highly polyploid and aneuploid nuclei. The effect after 120 hours was like the mass breakage which occurred spontaneously in *Lilium* endosperms.

In root tips the " primary effects " of X-rays which induce sub-chromatid breakage last only a few hours (La Cour and Rutishauser, 1953*a*). However, in these endosperms, sub-chromatid breakage occurred up to 120 hours after X-ray treatment. This indicates that the gross physiological disturbances produced by irradiations have a more lasting effect in this special and hybrid tissue.

9. DISCUSSION

(1) *Spontaneous chromosome breakage*

In these hyacinths the abnormalities in the endosperm begin with sticking of the anaphase chromatids. Anaphase movement results in mechanical breakage of the sticking chromatids. The breakage is recurrent owing to the bridge-breakage-fusion cycle. I consider that these primary bridges largely, if not wholly, result from sub-chromatid breakage and reunion.

Prolonged cold treatment induces similar errors in anaphase separation of the heterochromatic segments in *Trillium* (Darlington and La Cour, 1940; Kurabayashi, 1952). No heterochromatic segments were visible in these hyacinth endosperms, even after cold treatment, but if heterochromatin is associated with nucleic acid starvation the rapid mitotic rate of the endosperm may induce a condition approaching nucleic acid starvation and make the chromosomes liable to errors during reproduction.

The predominance of sub-chromatid breakage in the nucleolar arms and the control of these effects further indicates a relationship between [illegible] and chromosome reproduction. La Cour and Rutishauser (1953*a*) have shown that X-ray-induced sub-chromatid

breakage is confined to prophase and they suggest that these errors occur during reproduction (1953*b*). If these errors are reproductive errors then chromosome reproduction must occur during prophase for the subsequent mitosis.

The frequency of sub-chromatid bridges is correlated with the number of secondary SR bridges. Both increase with chromosome number of the particular endosperm in the same way as do spindle abnormalities (fig. 5). The similar behaviour of the chromosome and the spindle abnormalities suggests that the control of sub-chromatid breakage and reunion is exercised through the cytoplasm and is determined by nuclear-cytoplasmic relationships (*cf.* Mather, 1948).

(ii) *Endosperm failure*

We have seen that in hyacinths the cycle of chromosome breakage begins with spontaneous sub-chromatid breakage and persists as chromosome breakage through the bridge-breaking-fusion cycle.

Unless the system is buffered against the effects of loss, by balance between and within chromosomes, the abnormalities will induce more abnormalities and the endosperm will degenerate. In the more balanced complements the abnormalities increase only slowly and the young seeds survive. It seems that the most unbalanced endosperms are those where the extra chromosomes are all, or predominantly of the one type. I suggest that because of the lack of a buffering system the grossly unbalanced endosperms degenerate soon after fertilisation. Endosperm failure can thus be a selection mechanism eliminating unbalanced plants.

The effect of this spontaneous chromosome breakage on hyacinth endosperms thus seems to depend on three factors :—

(i) The number of normal nuclei present when breakage begins.

(ii) The chromosome balance in these nuclei.

(iii) The number of chromosomes in the nuclei.

Is the sequence of events, commencing with mitotic abnormalities and leading to endosperm failure and embryo abortion peculiar to hyacinths or can it be more generally applied ?

Many reports of endosperm failure include accounts of chromosome breakage and mitotic abnormalities (Beaudry, 1951 ; Boyes and Thompson, 1937 ; Brock, 1954 ; Cooper and Brink, 1944 ; Kihara and Nishiyama, 1932 ; Landes, 1939 ; Thompson and Johnson, 1945). These abnormalities frequently occur after crossing plants of different chromosome number. Abnormal endosperms occur in crosses where there are wide genetic differences (*e.g.* hybrids between species) and in crosses where there is little genetic difference between the parents (*e.g.* diploid × autotetraploid).

Even in the diploid-diploid crosses of the hyacinth there is considerable endosperm failure. Genetic character therefore influences the breakdown. But the failure increases where the chromosome

number of the endosperm is higher. The abnormalities must therefore be controlled at the chromosomal level as well as at the genic level.

Endosperm failure sometimes results from selfing plants which are normally outbreeders. This is essentially an incompatibility mechanism, but it has the important difference that it entails wastage of reproductive materials, and as such is inefficient. In *Medicago sativa* (Cooper and Brink, 1940) and *Secale cereale* (Landes, 1939) endosperm failure is combined with a slower pollen tube growth so that relatively few ovules are fertilised. However, in *Gasteria verrucosa* (Sears, 1937) and in *Theobroma cacao* (Knight and Rogers, 1953) there is no difference in the rate of pollen tube growth between compatible and incompatible pollinations. These are examples of sporaphytic control of incompatibility with the site of action in the fertilised ovule rather than in the style. They indicate also that the medium carrying the immediately determining factor is not the nucleus but the cytoplasm.

Brink and Cooper (1944) and Beaudry (1951) after crossing *Hordeum jubatum* by *Secale cereale* and *Elymus virginicus* by *Agropyron repens* found abnormalities in both endosperm and antipodals. They postulate a mechanism of endosperm failure involving the antipodals as intermediaries. No detailed studies have been made of the behaviour of the antipodal nuclei in the hyacinth crosses, but occasionally enlarged antipodal nuclei were observed. Thompson and Johnston (1945) failed to find any antipodal abnormalities associated with endosperm failure after crossing *Hordeum vulgare* by *Secale cereale*. In all the reported cases where abnormalities occurred in the antipodals some prior endosperm development had occurred so it seems likely that these abnormalities were a secondary rather than a primary effect.

Rappaport *et al.* (1950*a*, *b*) report seed failure in interspecific and diploid by autotetraploid *Datura* crosses. These seed failures are characterised by failure of the endosperm and embryo and proliferation of the endothelium, but the first abnormalities occur in the endosperm (Blakeslee *et al.*, 1953).

Thus we can conclude that the sequence of events, beginning with spontaneous chromosome breakage in the endosperm and culminating in endosperm failure and embryo abortion, occurs far more widely than in these hyacinths and is a common cause of seed failure after crossing.

10. SUMMARY

1. Diploid, hypo-triploid and triploid varieties of *Hyacinthus orientalis* with different numbers of L, L^n, M and S chromosomes, were used in crossing.

2. Rosalie, a trisomic for L^n, is a structural hybrid with inversions in two L chromosomes. The extra L^n chromosome appeared in only 16·5 per cent. of the cells at pollen grain mitosis.

3. Endosperm failure and subsequent embryo abortion occur after crossing. Spontaneous chromosome breakage and spindle abnormalities (resulting in polyploidy and aneuploidy) occur in these endosperms.

4. (i) The primary errors are breakage and reunion of sub-chromatids and possibly failure of the end gene to reproduce. These result in chromosome breakage; they lead to secondary errors in subsequent mitoses.

(ii) The centromere and the nucleolar organiser influence these sub-chromatid errors, which can therefore be related with chromosome reproduction. This influence is not found after X-raying.

5. (i) The range of chromosome balance in the developing embryos and endosperms agrees with that in the commercial hyacinth varieties.

(ii) In balanced forms some abnormalities occur but in unbalanced forms abnormalities accumulate and culminate in degeneration of the endosperm. They become more frequent as the chromosome number of the endosperm is increased.

(iii) An unusual internal balance in the hyacinth chromosomes is indicated by the unique transmission of a deficient chromosome by the haploid pollen; and by the healing against reunion after chromosome breakage, which occurs in these hyacinths but not in maize.

6. X-rayed and aged pollen induce abnormalities of mitosis in the endosperm.

7. The reduced spiralisation of the endosperm chromosomes reveals staining bodies in some of the nucleolar constrictions. Different varieties are homozygous or heterozygous for this condition.

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Plate I

Endosperms of hyacinth, various variety crosses. Fixation 12 to 16 days after pollination, in Carnoy fluid (6 : 3 : 1), stained with Feulgen reagent. Dissected and squashed in 45 per cent. acetic acid.

FIG. 1.—Sub-chromatid bridge at anaphase. ×900.

FIG. 2.—SR bridge in L^n. *Note* normal length of trabant top left. Short trabants in centre and long trabants on right demonstrate the effects of unequal breaking in bridge-breakage-fusion cycle. *Note* staining areas in nucleolar constriction, centre and right ; long non-staining constriction, top left. ×900.

FIG. 3.—Interlocked dicentric involving L and L at anaphase. ×900.

FIG. 4.—Ring chromosome involving L^n at anaphase. ×900.

FIG. 5.—Pollen grain mitosis in Rosalie showing bridge in L chromosome. Complement L3, L^n2, M2, S2. ×1350.

FIG. 6.—First anaphase of meiosis in Rosalie showing inversion crossing-over bridge and fragment in L chromosome and univalent L^n chromosome. ×1350.

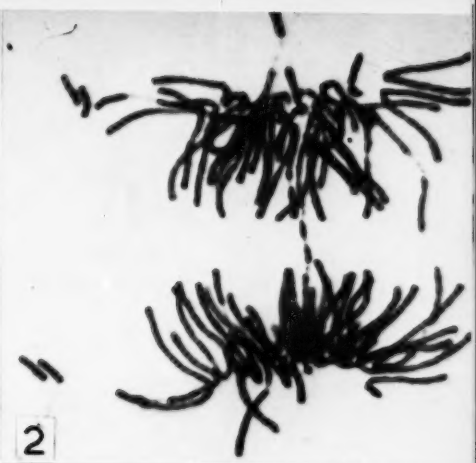

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Plate II

Endosperm of hyacinth, various variety crosses. Technique—as for plate I

FIG. 1.—Fusion of adjacent spindles at anaphase to produce highly polyploid nuclei. ×900.

FIG. 2.—Splitting of spindle at anaphase. This will result in aneuploid nuclei which if they are balanced will persist in endosperm (see fig. 4). ×900.

FIG. 3.—Two metaphases connected by unbroken bridge from previous division. This will probably result in restitution and high polyploidy. ×900.

FIG. 4.—Aneuploid anaphase L3, L^n2, M4, S2. This is one of the products of a split spindle (fig. 2) persisting in endosperm. ×900.

FIG. 5.—3x and 6x nuclei persisting in endosperm. Each nucleus carries the same dicentric and the 6x arose by fusion of spindles of adjacent nuclei (3x normal plus 3x including a dicentric). ×450.

FIG. 6.—Ovule 16 days after pollination with aged pollen (stored at 6° C. for 34 days). Unfertilised egg cell in prophase with a sperm nucleus beside it. Lower is the endosperm : one polyploid nucleus showing mass breakage. Antipodals not shown. In centre are extraneous nucellar nuclei. ×200.

FIG. 7.—Spontaneous chromosome breakage at the centromere (misdivision) of L^n chromosome. Nucleolar arms showing some centromere activity but delayed. Non-nucleolar arms acentric. ×900.

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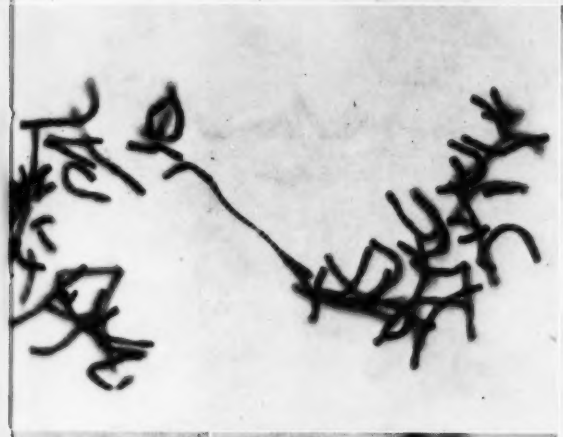

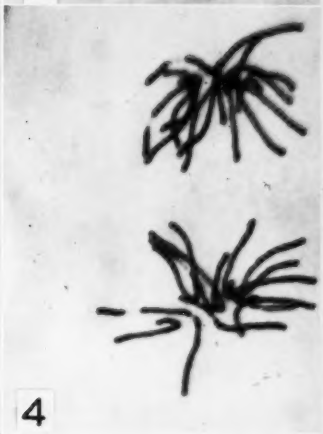

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A SURVEY OF THE GENETICAL BASIS OF HETEROSIS IN A VARIETY OF DIALLEL CROSSES

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1. INTRODUCTION

THERE are at present two principal hypotheses concerning the genetical basis of heterosis. The one holds that heterosis is caused by bringing together in the hybrid the dominant favourable genes of both parents (Jones, 1917). The second theory holds that heterozygosity *per se* is responsible for heterosis (East and Hayes, 1912 ; East and Jones, 1919 ; Shull, 1948). More recently East (1936) sought to explain heterosis in terms of the complementary action of alleles at the same locus, a phenomenon which has variously been described as " super-dominance " (Fisher, Immer and Tedin, 1932) and " overdominance " (Hull, 1945).

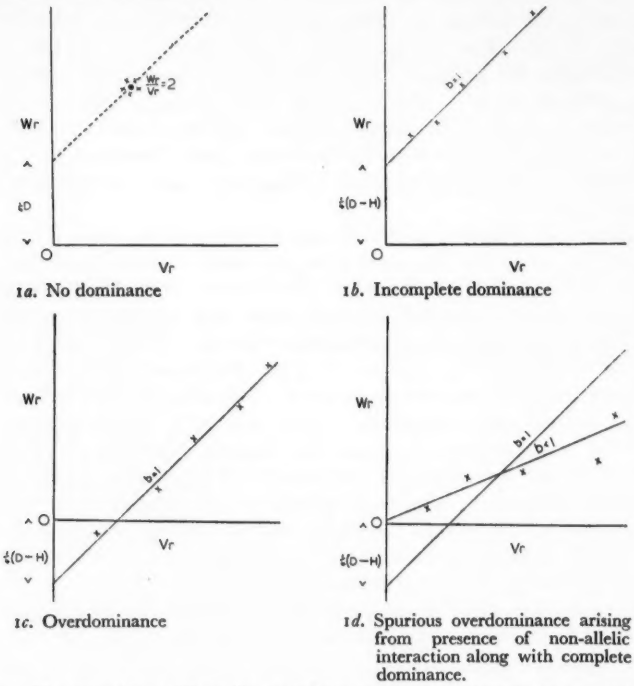
These two theories approach one another to the limit where it is impossible, from the practical point of view, to distinguish between alleles and closely linked genes. They hold important differences, however, for the practical breeder, since they propose two different methods of attaining maximum improvement. If the first theory is correct, it should be possible to gain maximum improvement by accumulating the maximum number of dominant favourable genes in the homozygous condition. The ease with which this can be achieved will be dependent on the linkage relations of the genes concerned and the degree of success will be dependent on the extent to which linkages of alleles in opposition are recombined. On the other hand, if the second theory is correct, maximum productiveness will follow only upon maximum heterozygosity, *i.e.* the heterosis is completely unfixable in the homozygous state.

Recently a method of analysing diallel crosses based on the methods described by Mather (1949) has been developed (Jinks and Hayman, 1953 ; Jinks, 1954*a*, 1954*b* ; Hayman, 1954) which allows one to discriminate between heterosis arising from interaction between alleles at one locus and between genes at different loci. The present paper sets out the results obtained on applying this method of analysis to a wide range of diallel crosses and related crossing programmes reported in the literature.

2. METHOD

In the present analysis we estimate from genetic parameters D, H_1, H_2 and F (see Jinks *loc. cit.* and Hayman *loc cit.*). Dominance is measured as $\frac{H_1}{D}$ which are weighted sums of h^2 and d^2 respectively (d and h being the additive and dominance components of variation),

the weighting being in favour of genes with both alleles represented equally in the parents. $\frac{H_2}{4H_1}$ provides an estimate of the mean value of uv, *i.e.* $\overline{uv}$ (with a maximum value of $\frac{1}{4}$ when $u = v = \frac{1}{2}$) and shows whether or not positive and negative alleles are present in equal proportions. This again is weighted, but this time in favour of genes showing the greater value of dominance, giving no information about genes showing no dominance. F is an indication of the relative frequencies of dominant and recessive alleles, being positive if there is an excess of dominants.



1*a*. No dominance

1*b*. Incomplete dominance

1*c*. Overdominance

1*d*. Spurious overdominance arising from presence of non-allelic interaction along with complete dominance.

FIG. 1.—The regression of Wr on Vr for various degrees of dominance (1*a*-1*c*). 1*d* shows how spurious overdominance may arise in these analyses as a result of non-allelic interaction.

Our test of the significance of the degree of dominance and its partitioning into true dominance and spurious dominance arising from non-allelic interaction is achieved by the regression of Wr on Vr (the covariance and variance of arrays respectively). In the absence of dominance there is no regression, all array variances and covariances being estimates of a single point where $\frac{Wr}{Vr} = 2$ (fig. 1*a*). Dominance uncomplicated by non-allelic interaction gives a regression

of unit slope which cuts the Wr axis on the positive side for incomplete dominance, at zero for complete dominance and on the negative side for overdominance (fig. 1*b* and 1*c*). The deviation of this point of intersection of the regression line and the Wr axis from the origin is equal to $\frac{1}{4}D-\frac{1}{4}H_1$, and hence its standard error can be calculated. The presence of non-allelic interaction leads to a deviation from unit slope giving a slope less than unity (fig. 1*c*), which should allow one to detect its presence irrespective of the degree of dominance.

3. EXAMPLES

(1) *Maize*

The most extensive of the sets of diallel crosses available in maize are those of Kinman and Sprague (1945). These consist of a 10 by 10 F_1 diallel and the means of F_2 families raised from these F_1s. These data have previously been analysed by Hull (1946) using the method of constant parent regression. This revealed significant overdominance in the F_1s but complete absence of dominance in the F_2s.

The re-analyses of these observations are given in columns 1 and 2 of table 1.

TABLE 1

Source of data	Kinman and Sprague, 1945		Nilsson-Leissner	Stringfield (Hull, 1946)
Generation	F_1	F_2	F_1	F_1
D	180·22	180·22	1775·10	260·19
H_1	1521·80	1349·54	27478·43	2605·15
H_2	1464·77	1201·42	25837·99	2530·74
F	+45·77	+88·32	+1102·72	+159·77
$\frac{H_1}{D}$	*8·44*	*7·49*	*15·48*	*10·01*
$\bar{uv}$	*0·241*	*0·224*	*0·235*	*0·243*
Hull's estimates of dominance	**2·91**	**0·00**	**2·05**	**1·88**
b wr/vr	0·676±0·108	0·707±0·114	0·258±0·179	0·737±0·122

In our present analyses F_1 and F_2 give high and consistent overdominance. The F_1 regression of Wr on Vr, the test for non-allelic interaction, is also significant, being less than 1 ($b = 0{\cdot}676\pm0{\cdot}108$). The main sources of interaction can be traced by omitting the progeny of the parental lines one at a time and in various combinations until an improvement of the regression slope is observed.

In this way it has been possible to show that in Kinman and Sprague's data parent B2 and, to a lesser extent, Hy and OhO7 are responsible for the bulk of the non-allelic interaction. Thus omitting the progeny of B2 gives a regression of $0{\cdot}754\pm0{\cdot}098$, while removing B2 and Hy or B2 and OhO7 gives regressions of $0{\cdot}788\pm0{\cdot}075$ and $0{\cdot}808\pm0{\cdot}086$ respectively. The latter is doubtfully no longer significantly different from a slope of 1 ($P = 0{\cdot}05$-$0{\cdot}02$).

While omitting the progeny of these three lines removes all the significant deviations arising from non-allelic interaction, it does not, of course, exhaust the sources of interaction present in the original parents. Each of the lines picked out must be interacting with at least one other parental line, that has been retained, to have been detected at all.

If we apply the scaling test (Mather, 1949) we find that we have not removed all the significant non-allelic interaction by omitting the progenies of parents B2, Hy and OhO7. For the purposes of the scaling test the diallel crosses are separated into the individual crosses, each consisting of the two parents, an F_1 mean and an F_2 family mean. The expectation in terms of d, h and the mid parent M are :—

$$\overline{P}_1 = M+\Sigma d \text{ } (\Sigma d \text{ is the balance of genes in opposition})$$
$$\overline{P}_2 = M-\Sigma d$$
$$\overline{F}_1 = M+\Sigma h$$
$$\overline{F}_2 = M+\Sigma \tfrac{1}{2}h$$

so that for each cross of the diallel table $\frac{1}{4}\overline{P}_1+\frac{1}{4}\overline{P}_2+\frac{1}{2}\overline{F}_1-\overline{F}_2 = 0$ in the absence of non-allelic interaction. One can, therefore, test for

	Cl14	R46	38·11	B2	WF9	OhO7	OhO4	WV7	K159
Hy	−	+	+	−	−	−	−	−	−
Cl14		+	+	−	−	−	−	−	−
R46			−	−	−	−	−	−	−
38·11				−	−	−	−	−	−
B2					+	+	+	+	−
WF9						−	−	−	−
OhO7							−	−	−
OhO4								−	+
WV7									+

FIG. 2

non-additivity of gene action by testing this equality. For greater accuracy, the modified scaling test proposed by Cavalli (1953) was used. The test consists of estimating by weighted least squares the three parameters Σd, Σh and M, taking as weights the reciprocals of the squared standard errors of each generation mean. These parameters can then be tested for consistency over generations, in this particular case, by a χ^2 for one degree of freedom.

Applying this test of additivity of gene action to Kinman and Sprague's data we find that there is significant deviation from simple additivity in 10 of the 45 crosses. These results are summarised in fig. 2, where + indicates non-additivity and − additivity on the present scale.

On this basis the parental lines can be divided into a number of

groups the members of which are similar in showing non-allelic interaction in crosses involving members of a second group (fig. 2).

These results tie up as well as can be expected with the F_1 regression test for non-allelic interactions. The latter not only picked out B2 as the main source of interaction, a result that is completely borne out by the scaling test, but actually accounted for 6 out of the 10 F_1 combinations showing non-allelic interaction.

We can now make estimates of the degree of dominance in one section of the data that contains no crosses exhibiting non-allelic interactions. This section comprises the four arrays whose common parents are Hy, Cl14, R46 and 38·11, and whose non-common parents are B2, WF9, OhO7, OhO4, WV7 and K159 for all the arrays. Using a modification of the diallel analysis described in detail under the section on flax (page 233) we can obtain two estimates of dominance $\frac{H_1}{D} = 2{\cdot}43$ and 2·71, which represent a fall in dominance of 71 per cent. and 68 per cent. respectively from the value found for the inclusive analysis (table 1). Our experience with another crop, *Nicotiana rustica* (Jinks, 1954*b*), is very similar. There the high spurious overdominance was not merely reduced but disappeared after omitting all crosses from the diallel analysis where non-allelic interaction had been demonstrated leaving only complete dominance, *i.e.* $\frac{H_1}{D} = 1$.

The alternative method of attempting to remove interaction, *i.e.* changing the scale, has proved unsuccessful. No one transformation has been found which will remove non-additivity in the crosses where it is present and maintain the *status quo* where it was formerly absent. For example, a log transformation removes all significant non-additivity in crosses where it was observed on the linear scale but a number of crosses where it was not previously observed, *e.g.* B2 × K159, WV7 × 38·11 and R46 × OhO4 now show significant non-additivity. Furthermore, the F_1 regression of Wr on Vr on the log scale differs significantly from unit slope ($b = 0{\cdot}694 \pm 0{\cdot}071$), again indicating non-allelic interaction. On the new scale the highest incidence of interaction is in arrays OhO4. It can be seen, therefore, that there is no prospect of obtaining an estimate of dominance unbiased by non-allelic interaction by this method.

The mean yield of F_1 families showing non-allelic interaction is 90·27 compared with 77·30 for the non-interacting F_1s, the mean of the parents giving rise to these F_1s being 29·49 and 27·91 respectively. On the average therefore, the F_1 families showing non-allelic interaction yield 13 bu/acre more than those showing no such interaction. It would thus appear that whereas general combining ability may be due to the operation of dominance in the F_1 families, non-allelic interaction must be implicated in the special combining ability which leads to the outstanding F_1 families.

It is impossible to assess from the data available how far the general combining ability is the result of heterozygosity *per se*. For one thing we can never be certain that we have removed all non-allelic interaction from data simply because we can no longer detect any significant deviations due to its action. It is, however, worth noting that an $\frac{H_1}{D}$ greater than 1 obtained in these analyses, even after removing all non-allelic interaction, is not automatically ascribable solely to over-dominance. Not only can linkage between genes in opposition give rise to spurious overdominance but it can also arise from the mere non-random association of genes in the parental lines. This is the general case of Jones' (1917) theory of heterosis. Thus if we have two genes A-a and B-b appearing only in the opposition associations, *i.e.* Ab and aB in the parental lines, the dominance ratio $\frac{H_1}{D}$ no longer estimates $\frac{\Sigma h_a^2+h_b^2}{\Sigma d_a^2+d_b^2}$ but $\frac{\Sigma(h_a+h_b)^2}{\Sigma(d_a-d_b)^2}$, which is, of course, an overestimate of the true degree of dominance.

TABLE 2

	Complete data	Omitting progenies of line 1
D	36·982	41·316
H_1	1527·111	1292·211
H_2	1432·062	1259·703
F	−0·676	+45·558
$\frac{H_1}{D}$	*41·293*	*31·276*
$\overline{uv}$	*0·234*	*0·244*
b	0·396±0·348	0·705±0·160

The analysis of two further sets of maize data (Nilsson-Leissner, 1927) and Stringfield (Hull, 1946) agree as far as they go with the situation found in the data of Kinman and Sprague. In the absence of further generations a detailed analysis of non-allelic interaction is not justified.

One further maize diallel has been analysed which, although only a 5 by 5 set of crosses, contains information regarding the incidence of non-allelic interaction and the past history of the inbred lines. This is contained in a paper by Rameshwar Singh (1953) and relates to his R×R crosses, the character under consideration once more being yield.

The relationship between the 5 parental lines can best be expressed if we number them 1 to 5 such that line 1 has no immediate past history in common with 2, 3, 4 or 5, whereas 2, 3 and 4 have one grandparent in common while 3, 4 and 5 have one parent in common. The analysis of the complete data is set out in table 2.

Reference to the Wr, Vr graph shows that the point for line 1 deviates most from the expected slope of 1. On omitting the progenies of this line from the analysis, there is a 25 per cent. drop in the degree of overdominance and a marked improvement in the Wr, Vr regression, although there is evidence that the non-allelic interaction has still not been wholly removed. The point for line 2 now deviates most from the expected slope, but the diallel is unfortunately too small to pursue the removal of non-allelic interaction any further. Even so a general picture of the situation is suggested, namely that in this set of crosses the intensity of non-allelic interaction can be related to the degree of relationship between the parental lines. Furthermore, our central theme is borne out by the fact that the omission of crosses showing non-allelic interaction leads to a fall in what can only now be described as spurious overdominance.

If we list the arrays in the order of the intensity of interaction displayed by these analyses, we find a marked positive correlation with the mean yield of their members (table 3).

TABLE 3

Arrays	F_1 yield
1	80·8
2	72·2
3, 4, 5	59·0

As in the data of Kinman and Sprague we find that the ability to produce outstanding F_1 yields, *i.e.* specific as opposed to general combining ability, are associated with the presence of non-allelic interaction.

(ii) *Egg plants*

The egg plant data are supplied by Gotoh (1953) and consist of a 5 by 5 diallel scored for a variety of characters. These observations have been previously analysed by Griffing's (1950) extension of Hull's constant parent regression technique.

The analyses of the various characters are given in table 4.

Using Griffing's analysis, Gotoh found it necessary to transfer the weight of fruit observations to a logarithmic scale but found the linear scale satisfactory for the other characters scored. Re-analysis of these data on the original scale by any of the scaling tests that are applicable to an F_1 diallel (Jinks and Hayman, 1953) shows no justification for rescaling. There is, however, extreme inequality of allele frequencies in the parent lines for weight of fruit and flowering time, *i.e.* F is almost as large as D and $\overline{uv}$ is much smaller than 0·25.

This would necessitate rescaling if Griffing's method of analysis is employed. In fact, Griffing's method is unable to distinguish between disturbances resulting from non-random distribution of alleles in the parental lines and those arising from genic interactions.

Weight of fruit, and shape of fruit, both show significant but incomplete dominance while the regressions of Wr on Vr all agree with a slope of 1, *i.e.* there is no suggestion of non-allelic interaction for these characters. Flowering time, on the other hand, shows

TABLE 4

	Weight of fruit	Shape of fruit index	Flowering time	Bunchiness of fruit
D	24,460·70	6·064	31·51	1·914
F	−21,260·16	−0·565	+18·61	+0·326
H_1	12,058·58	0·939	20·02	0·080
H_2	7,412·13	0·767	4·55	...
$\frac{H_1}{D}$	*0·493*	*0·155*	*0·635*	*0·042*
$\overline{uv}$	*0·155*	*0·204*	*0·057*	...
$b_{Wr/Vr}$	1·029±0·026	1·010±0·036	0·855±0·228	...
$\frac{1}{4}(D-H_1)$	3,100·53±502·67 (D>H_1)	1·281±0·010 (D>H_1)	2·873±2·250 (D = H_1)	...

complete dominance, but again gives no suggestion of non-allelic interaction.

The other character scored, bunchiness of fruit, consisted of only one array, *i.e.* one parental line crossed to four others. The appropriate formulæ for this type of analysis are as follows :

$$V_{OLO} = D$$
$$V_r = \tfrac{1}{4}D + \tfrac{1}{4}H_1 + \tfrac{1}{4}F_r \text{ where } F_r = \Sigma 8uvdh$$
$$W_r = \tfrac{1}{2}D + \tfrac{1}{4}F_r$$

TABLE 5

	Yield	Height	Sowing to harvesting
D	60,635·61	74·33	1·314
H_1	554,104·99	232·40	1·237
F	+148,862·50	+128·69	−0·500
$\frac{H_1}{D}$	*9·138*	*3·126*	*0·942*

This analysis gives no estimate of H_2 and hence of $\overline{uv}$, nor a test of non-allelic interaction when confined to F_1 data only. It does permit one to estimate the degree of dominance without, however, providing a test of significance from the regression of Wr on Vr. In this particular case dominance is negligible (table 4).

One further set of crosses has been analysed consisting of a single array, *i.e.* 1×9 crosses. This gives no information about the incidence of non-allelic interaction, but it is of interest in so far as it contrasts

with the other results in showing overdominance, spurious or otherwise, for some of the characters scored. These data are given in a paper by Kakisaki (1931) and include observations on yield, final height and the period from sowing to harvesting. The analyses are set out in table 5.

(iii) *Galeopsis*

It is possible to extract some complete diallel tables from the results of numerous crosses made by Hagberg (1952*a*, 1952*b*) within

TABLE 6

	1947	1948	1950	*G. bifida* × *tetrahit*, 1947
D	138·917	170·917	251·300	95·000
H_1	19·750	41·333	29·660	11·500
H_2	13·521	29·938	25·120	9·083
F	+6·916	+10·750	−53·200	−10·667
$\frac{H_1}{D}$	*0·142*	*0·242*	*0·118*	*0·121*
$\frac{\overline{uv}}{}$	*0·171*	*0·181*	*0·212*	*0·198*
$b\frac{Wr}{Vr}$	0·910±0·018	0·926±0·030	1·079±0·113	0·939±0·167
$\frac{1}{4}(D-H_1)$	29·79±0·60 (D>H_1)	32·39±2·43 (D>H_1)	55·41±2·28 (D>H_1)	20·88±1·10 (D>H_1)

and between varieties of *Galeopsis tetrahit* and *bifida* carried out over a number of seasons. The largest of these tables for flowering time is provided by a 5 by 5 F_1 diallel within varieties of *G. tetrahit*. Two further 4 by 4 diallel sets of crosses involving the same parental lines can be extracted from the 1947 and 1948 data which have three parents in common with the 1950 diallel. The analyses are given in table 6.

TABLE 7

	Single array	3×3 diallels I	II
D	34·629	43·333	19·000
H_1	58·334	281·891	12·556
H_2	...	201·216	11·112
F	−57·016	+92·216	+1·333
$\frac{H_1}{D}$	*1·685*	*6·505*	*0·608*
$\frac{\overline{uv}}{}$	...	*0·179*	*0·221*
b Wr/Vr	...	0·993±0·375	0·953±0·206
$\frac{1}{4}(D-H_1)$	...	−59·65±5·52 (D<H_1)	1·61±0·58 (D = H_1)

Over all years there is significant but incomplete dominance, with no suggestion of non-allelic interaction.

It is impossible to extract diallels larger than 3 by 3 from the height data. It is possible, however, to extract a single array (1×11) and two 3 by 3 diallels chosen from amongst these 12 parents. The analyses are given in table 7.

The analysis of the single array shows slight overdominance which unfortunately cannot be tested for significance since none of the tests is applicable. Of the selection of two 3 by 3 diallels from amongst the parents making up the single array, one shows significantly high overdominance while the other shows no significant deviation from complete dominance. There is, however, only one degree of freedom for testing the WrVr regression for non-allelic interaction and neither regression is significant. Although a high intensity of non-allelic interactions can give rise to a non-significant regression, we cannot with any confidence ascribe it to this cause in the present case because of the paucity of the material.

The only remaining diallel is a 4 by 4 set of crosses grown in 1947, using two varieties of *G. tetrahit* and two of *G. bifida* as parents. The analysis of the times of flowering show significant but incomplete

TABLE 8

	Height	Leaf length
D	100·25	6·340
H_1	111·73	1·424
H_2	84·37	1·281
F	+75·62	+0·220
$\frac{H_1}{D}$	*1·115*	*0·225*
$\overline{uv}$	*0·189*	*0·225*
b Wr/Vr	1·142±0·221	0·982±0·068
$\frac{1}{4}(D-H_1)$	−2·87±5·95 ($D = H_1$)	1·23±0·05 ($D > H_1$)

dominance, while the WrVr regression does not differ significantly from a slope of 1 (table 6). Once again we have neither overdominance nor non-allelic interaction.

(iv) *Nicotiana rustica*

Apart from the results which have already appeared on diallel crosses within this species (Jinks, 1954*b*) a 4 by 4 diallel carried out by H. H. Smith (1952) is presented in a form amenable to further analysis.

Amongst the characters scored are height and leaf length, which have added interest in that they are two of the characters used in our own diallel. The analyses are given in table 8.

For height we find there is complete dominance and no suggestion of non-allelic interaction. Although we found overdominance and significant non-allelic interaction in our own data it will be recalled that only complete dominance remained after omitting the crosses showing non-allelic interaction.

The absence of non-allelic interaction is confirmed for one cross of the diallel set by a joint F_2 and backcross scaling test carried out

by Smith, which revealed no deviation from simple additivity of gene action. Furthermore, all possible double crosses could be predicted with a high degree of precision by Jenkins' method (1940) so that there could be no marked non-allelic interactions between the genes.

The situation found here for leaf length is also similar to our own results, *viz.* incomplete but significant dominance with no non-allelic interaction.

(v) *Flax*

Carnahan (1947) has reported data on flax yields that involve a common variant on the diallel cross technique. It consists of crossing a collection of new varieties with a collection of tester stocks. Thus, instead of giving a diallel table with n^2 observations we have a comparable table containing n_1n_2 observations where n_1 and n_2 are the number of tester and new varieties respectively.

In the absence of reciprocal differences the statistics obtained from the analysis of rows and columns of a diallel table are estimates of the same thing. In the tester cross type of table, however, the analysis of rows and columns give different estimates depending on the distribution of alleles in the n_1 and n_2 sets of parental lines.

If the distribution of the two alleles A and a in the n_1 lines is u and v while in the n_2 lines it is s and t respectively, we can by analogy with the diallel analysis obtain the following expectations for the statistics :

Statistics	n_1 parents	n_2 parents
V_{OLO}	$\Sigma 4uvd^2$	$\Sigma 4std^2$
V_{1L1}	$\Sigma uvd^2+uvh^2-2uvs-\overline{tdh}$	$\Sigma std^2+sth^2-2stu-\overline{vdh}$
W_{OLO1}	$\Sigma 2uvd^2-2uvs-\overline{tdh}$	$\Sigma 2std^2-2stu-\overline{vdh}$
V_{LO1}	$\Sigma uvd^2+uvh^2-4uvsth^2$ $+2stu-\overline{vdh}-2ut+vs.us-\overline{tvdh}$	$\Sigma std^2+sth^2-4uvsth^2$ $+2uvs-\overline{tdh}-2ut+vs.us-\overline{tvdh}$

From these statistics we can estimate D, H_1 and F defined as follows :

Parameter	n_1 parents	n_2 parents
D . . .	$4\Sigma uvd^2$	$4\Sigma std^2$
H_1 . . .	$4\Sigma uvh^2$	$4\Sigma sth^2$
F . . .	$8\Sigma uvs-\overline{tdh}$	$8\Sigma stu-\overline{vdh}$

The estimates of these parameters are given in table 9.

The two estimates of the degree of dominance differ as a result of the different weights of the *d*'s and *h*'s arising from dissimilar distributions of alleles in the two sets of parental lines. They agree, however, in showing overdominance. The test for non-allelic interaction, which is the same as in the diallel cross, gives no significant regression in both cases.

Only two genetical causes can give rise to a non-significant regression ; absence of dominance and non-allelic interaction (fig. 1). The first is belied by the high degree of dominance obtained from the $\frac{H_1}{D}$ ratios. Furthermore, there is significant heterogeneity among the variances of arrays on Stevens' test (1936) which is incompatible with no dominance (Jinks, 1954*b*). We can only conclude, therefore, that the disturbances in the regression analyses are the direct result of non-allelic interaction. Evidence in favour of this view is supplied by re-analysis of three arrays which deviated least from the best fitting regression line of unit slope. Two of these are from the n_2 parents and the other from the n_1 group. These gave dominance ratios of $\frac{H_1}{D} = 1{\cdot}800$ and $1{\cdot}019$ respectively, which represent a 43·5 and 92·8 per cent. drop in dominance over the inclusive analysis (table 9). This is more than suggestive that non-allelic interaction is again implicated in the high degree of " overdominance ".

TABLE 9

	n_1 parents	n_2 parents (tester stocks)
D . . .	6·917	3·333
H_1 . . .	22·084	46·500
F . . .	+7·167	−2·334
$\frac{H_1}{D}$ . . .	*3·193*	*13·950*
b Wr/Vr . .	0·288±0·154	0·087±0·035

4. DISCUSSION

The first and most important fact that emerges from these analyses is that in no cases have we found evidence of overdominance without also finding evidence for the simultaneous presence of non-allelic interaction. Where it has been possible to test for the presence of interactions in more advanced generations by means of scaling tests they have without fail confirmed our earlier findings.

It has not been possible in all cases to pursue the coincidence of overdominance and non-allelic interaction further than their detection, mainly because of the paucity of the material where the diallel involves small numbers of parental lines.

In the one case where a complete analysis has been possible, namely our own diallel crosses in *N. rustica*, the removal of all crosses showing significant non-allelic interaction as detected by the WrVr regressions and joint F_2 and backcross scaling tests led to a fall in the dominance ratio to a value of $\frac{H_1}{D} = 1$. Thus the overdominance can be related to a spurious inflation, in material showing only complete dominance, as a result of non-allelic interaction. The less complete analysis of maize (Kinman and Sprague's data) also showed a fall in the dominance ratio (in this case a 70 per cent. drop) after omitting all crosses from the analysis showing non-allelic interaction on the WrVr regression and the F_2 scaling test, although in this case significant overdominance, at a much lower level, still remained, as did some evidence of interaction. For the rest of the data we cannot make such sweeping claims with the same degree of confidence because of the necessarily incomplete nature of some of the analyses. But we can with some confidence ascribe the phenomenon described as special combining ability to the direct outcome of non-allelic interaction, while general combining ability can be related to the expression of dominance.

Whether true overdominance, in the sense used by East (1936) and Hull (1945) is operative in any of the data is not made clear from the analyses described here. We can say that in no case is this the sole explanation. We can further say, with some confidence, that it plays no part in any of the *N. rustica* data so far analysed, while its contribution to the total heterosis in the maize data, at the most can only be of the same order as that of non-allelic interaction and is probably much less. And it is the heterosis produced by the latter, *i.e.* specific combining ability, that is of primary importance to the maize breeder of to-day. Until such time as observations on segregating generations are available for analysis in the way described by Mather (1949) and Mather and Vines (1952), which would reveal the role of association and linkage in the creation of spurious overdominance, can we hope to be in a position to evaluate the role of true overdominance.

Two further breakdowns of these results are possible, the first relating to the breeding systems and the second to the type of character whose inheritance is under investigation (table 10).

As we pass from outbreeders to inbreeders there is no marked falling off either in the degree of dominance or in the incidence of non-allelic interaction. Moving down the table, however, there is a marked falling off of both. Any attempt to explain this observation must take into account past selection of the character under consideration. Our main problem here is to try and relate the two.

Flowering time, which exhibits neither high dominance nor non-allelic interaction in any of the data analysed, is obviously restricted in its variation as a consequence of its close correlation with time of

season. That is to say, the extremes of environment within which flowering and hence seed maturation may be brought to a successful conclusion is very limited. Within such a restraint, heterosis of the magnitude met with in characters included under the general heading of vigour would be disastrous for the plant exhibiting it as one or other of the processes leading from flowering to seed maturation would be jeopardised by the prevailing environmental conditions. The genetic system would be further reinforced by the existence of linkage between genes controlling characters such as flowering time as found, for example, in *N. rustica* (Mather and Vines, 1952 ; Jinks, unpublished).

TABLE 10
Breeding system

Character		Outbreeder → Inbreeder				
		Maize	*N. rustica*	Egg plants	Flax	*Galeopsis* spp.
YIELD	Dominance ratio	8-40	...	0·5-9	3-14	...
	NON-ALLELIC INTERACTION	+	...	− or +	+	...
HEIGHT	Dominance ratio	...	1·4	3	...	0·6-7
	NON-ALLELIC INTERACTION	...	+ and −	− or +	...	+ and −
LEAF CHARACTERS	Dominance ratio	...	0·2-0·6	...	...	...
	NON-ALLELIC INTERACTION	...	+ and −	...	...	...
FRUIT SHAPE	Dominance ratio	...	...	0·2	...	...
	NON-ALLELIC INTERACTION	...	...	−	...	...
FLOWERING TIME	Dominance ratio	...	0·5	0·6-0·9	...	0·2
	NON-ALLELIC INTERACTION	...	−	−	...	−

In fact the existence of such phenomena as photoperiodism suggests that there may well be a tying together of genes controlling characters subject to such restraints into supergenes behaving as single switches in response to the right environment. Low dominance, absence of non-allelic interaction and correlated inheritance through linkage would all work towards one end, namely the production of progeny whose flowering behaviour is similar to its own successful parents. This could go a long way towards explaining the observations in table 10, but we must reserve final judgment until such time as character analyses over all breeding systems are available in order to avoid possible confounding between the two.

When we turn to shape indices, characterised as ratios of lengths

to breadths, etc. we meet with restraints, which while different in origin will nevertheless have similar effects. In this case heteroses of the magnitude met with in vigour characters would result in a non-functional or mechanically unstable organ.

Undoubtedly, physical and physiological restraints of some type limit the range of expression of every type of character. In some, however, notably the two discussed above, the limits are at once more apparent and more finely balanced. While under strong selection most types of restraint can be broken, except insofar as pleiotropy is operative, they are unlikely to exist in this form in wild populations or unselected material. But it is in vigour characters, where restraints are expected to be less rigid, more than in any of the others, that artificial selection has been sufficiently intense to break down any restraints existing in the wild because of their immediate economic importance.

5. SUMMARY

Published data of a number of diallel crosses and related crossing programmes employing such varied material as *maize, flax, egg plants, Galeopsis* spp. and *Nicotiana rustica* have been analysed by a method which can discriminate between heterosis arising from interaction between alleles at one locus, *i.e.* overdominance and that arising from interaction between non-allelic genes. These analyses show that :

(i) Wherever we find overdominance we also find non-allelic interaction.

(ii) Re-analysis of the data after omitting all crosses showing significant non-allelic interaction leads in all cases to a drop in the apparent degree of overdominance and in one case to the complete disappearance of the spurious overdominance.

(iii) Specific combining ability is always associated with the presence of non-allelic interaction while general combining ability is the outcome of uncomplicated dominance.

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MULTIPLE MUTATION IN *CHLAMYDOMONAS REINHARDI*

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1. INTRODUCTION

THE rate of natural or induced mutation at most loci is so low that it is uncommon to find an individual in which simultaneous mutation at more than one locus has occurred. This paper will describe the genetical analysis of a mutant clone of *Chlamydomonas reinhardi* which seems to have arisen by simultaneous mutation at three loci.

2. MATERIALS AND METHODS

C. reinhardi is a heterothallic, unicellular green alga. Genetical evidence indicates that the vegetative cells are haploid. When two clones of opposite mating-type are mixed together under suitable conditions, sexual fusion occurs and zygotes are formed. The zygote represents the only diploid stage in the life-cycle, and meiosis occurs when the zygote germinates. The strains used in this work were isolated from soil by Professor G. M. Smith of Stanford University, California, who kindly supplied the author with cultures.

In genetical experiments with *C. reinhardi* the techniques used have been modified from those of Smith and Regnery (1950), and will be described in detail elsewhere. The life-cycle can be completed within three weeks. Zygotes germinate readily and the products of germination, usually four or eight cells, can be isolated. The tetrad is not ordered. The majority of zygotes germinated produce eight cells, but since these represent four pairs all zygotes can be treated as though they produce four cells.

3. THE *EYELESS* TYPE

Cultures of wild-type cells in liquid medium were irradiated with ultra-violet light in order to produce mutations, after which the cells were spread over the surface of nutrient agar plates. The plates were kept in continuous light and after several days colonies, each produced from a single irradiated cell, were visible on the agar surface. Cells from these colonies were examined under the microscope for morphological differences from wild-type, and in one clone it was found that the characteristic red eyespot was lacking. This character has remained constant through repeated sub-cultures and is given the name *eyeless* (symbol *ey*).

The original eyeless clone was of *minus* mating-type, and it was crossed with the wild-type *plus* strain. The products of 58 zygotes were examined for presence of the eyespot and in all cases there was 2 : 2 segregation for presence and absence. It was therefore concluded that absence of the eyespot was due to a single gene difference.

From the progeny of this first cross, an eyeless clone of *plus* mating-type was selected and crossed with wild-type *minus*. The products of 36 zygotes were examined and again showed regular 2 : 2 segregation for presence and absence of the eyespot.

4. THE *SMALL-COLONY* TYPE

A striking feature of progenies from both these crosses was the appearance of certain colonies which were smaller and paler than normal. The difference in size and colour was quite unmistakable. Normal colonies were about 2 mm. in diameter after a week's growth and were a rich dark green colour; small colonies, however, rarely exceeded 0·5 mm. in diameter and were pale yellow. Each set of four colonies from a single zygote included either two, one or none which were of this type. In table 1 the zygote progenies from the two crosses described above have been classified according to the ratio of normal to small colonies.

TABLE 1

Results of crosses between eyeless (ey) *and wild-type* (ey^+)

Number and description of cross	Number of zygotes analysed	Number of zygotes producing normal and small colonies in the ratio		
		4 : 0	3 : 1	2 : 2
1. $ey- \times ey^+ +$ . . .	58	2	54	2
2. $ey+ \times ey^+ -$ . . .	36	2	32	2
Totals . . .	94	4	86	4

As was mentioned above, all zygote progenies were examined for presence or absence of the eyespot. Not only did each progeny show 2 : 2 segregation for this character, but the small colonies invariably consisted of cells lacking eyespots. It should be noted, however, that colonies of cells without eyespots were not necessarily small.

These observations led to a hypothesis which was then tested by further crosses. The hypothesis is that a gene *small-colony* (symbol *sc*) is responsible for reduced colony size, and that this gene is suppressed by the wild-type allele at the eyeless locus (ey^+) but not by the mutant allele (*ey*). If this is so, the parental genotypes in cross no. 1 (table 1) may be amplified to read as follows:

$$ey\ sc^+ - \times ey^+\ sc +$$

Zygotes from this cross would yield progenies of three types (ignoring for the moment the inheritance of mating-type):

1. Segregation into the two parental genotypes;

$ey^+\ sc$ normal colony
$ey^+\ sc$,, ,,
$ey\ sc^+$,, ,,
$ey\ sc^+$,, ,,

2. Segregation into four genotypes ;

ey$^+$ *sc*$^+$ normal colony
ey$^+$ *sc* ,, ,,
ey *sc*$^+$,, ,,
ey *sc* small colony

3. Segregation into two non-parental genotypes ;

ey$^+$ *sc*$^+$ normal colony
ey$^+$ *sc*$^+$,, ,,
ey *sc* small colony
ey *sc* ,, ,,

These three types of segregation would account for the three types of progeny obtained from crosses 1 and 2 (table 1).

Experiments designed to test the hypothesis were carried out using sets of clones from the zygote progenies of cross no. 1. The first set, from zygote O_4, consisted of the following eight clones :

(a) and (g) *ey*$^+$+ normal colony
(b) and (h) *ey*$^+$− ,, ,,
(c) and (d) *ey* − ,, ,,
(e) and (f) *ey* + ,, ,,

According to the hypothesis this set belongs to type 1 (see above), and all clones carrying the *ey*$^+$ gene should also carry *sc*, though this is not expressed. If each clone with eyespots were crossed with an eyeless strain there should be produced in each case some recombinant clones with small colonies. Crosses of this sort were made, with the results shown in table 2 (crosses 3-6).

Zygote S_2 gave rise to the following eight clones :

(a) and (c) *ey* (−)* small colony
(b) and (g) *ey* − normal colony
(d), (e), (f) and (h) *ey*$^+$ + ,, ,,

This set of clones shows a 3 : 1 ratio of normal and small colonies, and so belongs to type 2. Clones (d), (e), (f) and (h) are phenotypically all alike, but according to hypothesis two of them should carry *sc* and two *sc*$^+$. By crossing them with an eyeless strain the genotypic difference should become apparent ; recombinant clones with small colonies should be produced from only two of the crosses. All four crosses were made and the results are shown in table 2 (crosses 7-10).

Zygote V_4 gave rise to the following eight clones :

(a) and (h) *ey* (−) small colony
(b) and (c) *ey*$^+$ − normal colony
(d) and (e) *ey* + ,, ,,
(f) and (g) *ey*$^+$ + ,, ,,

This set also belongs to type 2, and of the four clones with eyespots

* Brackets around the mating-type symbol indicate that in this case mating-type was determined by inference. In only a few cases has it been possible to determine directly the mating-type of small-colony clones.

only two are expected, by hypothesis, to carry *sc*. In this case there is the further expectation that the two carrying *sc* are either (b) and (c) or (f) and (g) since mating-type tests have shown that these are sister clones. Results of the crosses made with these clones are shown in table 2 (crosses 11-14).

Zygote EE1 gave rise to the following eight clones :

(a) and (b)	*ey*+ —	normal colony
(c) and (d)	*ey*+ +	,, ,,
(e), (f), (g) and (h)	*ey*	small colony

This set belongs to type 3. All eyeless clones have small colonies, and it is therefore to be expected that none of the clones with eyespots

TABLE 2

Results of crosses made to study the inheritance of the small-colony type

Number and description of cross*	Number of zygotes analysed	Number of zygotes producing normal and small colonies in the ratio		
		4 : 0	3 : 1	2 : 2
3. O4a×*ey*−	*5*	1	4	0
4. O4b×*ey*+	*12*	0	11	1
5. O4g×*ey*−	*9*	1	8	0
6. O4h×*ey*+	*10*	1	7	2
7. S2d×*ey*−	*23*	23	0	0
8. S2e×*ey*−	*14*	1	13	0
9. S2f×*ey*−	*14*	1	12	1
10. S2h×*ey*−	*9*	9	0	0
11. V4b×*ey*+	*37*	37	0	0
12. V4c×*ey*+	*11*	11	0	0
13. V4f×*ey*−	*15*	3	11	1
14. V4g×*ey*−	*15*	4	11	0
15. EE1a×*ey*+	*20*	20	0	0
16. EE1b×*ey*+	*25*	25	0	0
17. EE1c×*ey*−	*7*	7	0	0
18. EE1d×*ey*−	*12*	12	0	0

* The first parent in cross no. 3 was clone (a) from zygote O4 ; this has been written as O4a. Similar abbreviation has been used in describing other parent strains.

carry *sc*. If this were so none of the clones with eyespots should produce small-colony clones when crossed with an eyeless strain. The results of such crosses are shown in table 2 (crosses 15-18).

Table 2 shows that all crosses yielded results in agreement with the hypothesis, and hence there is evidence for the existence of the *sc* gene and for its behaviour in the manner postulated.

The *ey* and *sc* genes do not appear to be linked. Table 1 shows that parental and non-parental two-type segregations are equally frequent in crosses segregation for alleles at both loci, and this is as expected when dealing with two unlinked loci.

The frequency of four-type segregations (91·49 per cent.) significantly exceeds 66·67 per cent. This indicates that there is more than 50 per cent. crossing-over between one of the loci and its centromere resulting in this locus showing more than 66·67 per cent. second-division segregation (Whitehouse, 1949). Since there is evidence that the *ey* locus is close to its centromere (see p. 245) it is probably the *sc* locus which has this high frequency of second-division segregation.

5. THE *SLOW-GROWTH* TYPE

Table 2 described the progenies from certain zygotes as consisting of normal colonies only. This means that all the colonies were dark green in colour and capable of reaching a diameter of at least 2 mm.; there is no possibility of classifying any of these as a small-colony

TABLE 3

Further classification of progenies from crosses which were not segregating for small-colony

Number and description of cross	Number of zygotes analysed	Number of zygotes producing full-sized and slow-growing colonies in the ratio		
		4 : 0	3 : 1	2 : 2
7. S2d×*ey*−	*23*	23	0	0
10. S2h×*ey*−	*9*	9	0	0
11. V4b×*ey*+	*37*	21	2	14
12. V4c×*ey*+	*11*	4	0	7
15. EE1a×*ey*+	*20*	0	0	20
16. EE1b×*ey*+	*25*	0	0	25
17. EE1c×*ey*−	*7*	7	0	0
18. EE1d×*ey*−	*12*	12	0	0

type. Nevertheless in certain of the progenies some variation in colony size was apparent. In these cases either four or occasionally only two colonies from the set of eight were noticeably smaller than their fellows, having only about three-quarters their diameter. It was therefore possible to amplify the classification of zygote progenies to include this character, which is referred to as *slow-growth*. In table 3 the progenies from those crosses not also segregating for small-colony are classified in this way.

Since each of the crosses in table 3 had one parent with the eyespot and one without, each progeny showed 2 : 2 segregation for presence and absence of the eyespot. When this was confirmed by examination of cells under the microscope it was found that all slow-growing clones lacked eyespots, though not all eyeless clones were slow-growing. This is a precisely similar situation to that found with small-colony clones, and a similar explanation appeared probable.

The recognition of the slow-growth character prompted a closer examination of growth-rate in the two eyeless strains which have been used throughout this work. Whenever a cross has involved an eyeless strain of *minus* mating-type, a subculture from the original mutant strain has been used. Colonies of this strain cannot be distinguished from wild-type colonies except by examination of individual cells under the microscope. The eyeless strain of *plus* mating-type used in cross no. 2 and subsequently, was selected from the progeny of cross no. 1. By comparison with wild-type colonies grown under similar conditions it has now been established that this eyeless *plus* strain shows slow-growth, though this passed unnoticed at the time of its selection. Since the progeny of cross no. 1 was segregating for small-colony, the presence of the less spectacular character, slow-growth, escaped attention.

In planning crosses to explain the inheritance of slow-growth it was postulated that a gene (symbol *sg*) causes slow growth in presence of the eyeless gene but is suppressed by its wild-type allele. On the basis of this hypothesis the crosses dealt with in table 3 are explained as follows :—

Crosses 7 and 10 : Neither parent carried *sg*, so no slow-growing clones appeared in any zygote progenies. Both crosses may therefore be written :

$$ey^+\ sg^+ \times ey\ sg^+$$

Crosses 11 and 12 : The eyeless parent carried *sg* but the other did not. In those progenies where *ey* and *sg* remained associated, slow-growing clones appeared ; but in others *sg* was recombined with its suppressor ey^+, and slow-growth was not expressed. Both crosses may be written :

$$ey^+\ sg^+ \times ey\ sg$$

Crosses 15-18 : Zygote EE1 was heterozygous for the *sg* locus, so that clones (a) and (b) received *sg* but clones (c) and (d) received the alternative allele sg^+. Clones (a) and (b) were crossed with eyeless *plus* carrying *sg*, so that all zygotes in these crosses (nos. 15 and 16) were homozygous for *sg* and all progenies included slow-growing clones. Crosses 15 and 16 may therefore be written :

$$ey^+\ sg \times ey\ sg$$

Clones (c) and (d) were crossed with eyeless *minus*, so that all zygotes were homozygous for sg^+ and no slow-growing clones were produced. Crosses 17 and 18 may be written :

$$ey^+\ sg^+ \times ey\ sg^+$$

According to the hypothesis all zygotes from cross no. 11 (table 3) have the genotype $ey^+\ sg^+/ey\ sg$. In the case of zygotes which produced eight full-sized colonies, the clones with eyespots should have the genotype $ey^+\ sg$. If these clones were crossed with eyeless *minus* (carrying sg^+) the progenies should include some recombinant clones of genotype *ey sg* which are slow-growing. Suitable clones of *plus* mating-type were selected from the progeny of cross no. 11 and crossed with eyeless *minus* ; the results are shown in table 4 (crosses 19 and 20).

Other zygotes from cross no. 11 produced four full-sized colonies

and four which were slow-growing. In these cases the full-sized colonies are postulated to have the genotype *ey*$^+$ *sg*$^+$, and when crossed with eyeless *minus* they should not yield any slow-growing clones at all. Suitable clones of *plus* mating-type were selected and crossed with eyeless *minus* ; the results are shown in table 4 (crosses 21 and 22).

All zygotes from cross no. 15 (table 3) are expected by hypothesis to have the genotype *ey*$^+$ *sg*/*ey sg*, and all clones in their progenies should carry *sg*. Those with eyespots have full-sized colonies, but when crossed with eyeless *minus* they are expected to yield some recombinant, slow-growing clones of genotype *ey sg*. Crosses of this kind were made with the results shown in table 4 (crosses 23 and 24).

All of the results in table 4 are consistent with expectations. Slow-growth is thus similar to small-colony in its relation to eyeless.

Progenies have been examined from 151 zygotes heterozygous at the *ey* and *sg* loci. They consist of 59 showing parental two-type,

TABLE 4

Results of crosses made to study the inheritance of slow-growth

Number and description of cross	Number of zygotes analysed	Number of zygotes producing full-sized and slow-growing colonies in the ratio		
		4 : 0	3 : 1	2 : 2
19. 35·5×*ey*− . . .	34	11	0	23
20. 35·7×*ey*− . . .	22	6	1	15
21. 35·1×*ey*− . . .	21	21	0	0
22. 35·2×*ey*− . . .	13	13	0	0
23. 35·11×*ey*− . . .	24	8	4	12
24. 35·12×*ey*− . . .	24	10	2	12

83 non-parental two-type and 9 four-type segregations. The two classes of two-type segregations are expected to be equally frequent if the loci are unlinked. There is here a significant deviation from a 1 : 1 ratio but it is in the opposite direction to that expected if the loci are linked. It is concluded that *ey* and *sg* are not linked, but some mechanism may exist whereby the progeny of certain crosses includes a high frequency of non-parental combinations.

The low frequency of four-type segregations (5·96 per cent.) indicates that both loci are close to their centromeres and therefore show a low frequency of second-division segregation.

6. DISCUSSION

Since the original mutant clone produced colonies indistinguishable from those of the wild-type except by microscopic examination of individual cells, it can be established that it had the genotype

ey sc$^+$ *sg*$^+$ —. In cross no. 1 (table 1) this strain was crossed with the wild-type strain of *plus* mating-type and the progeny included some clones showing the small-colony character and others showing slow-growth (see p. 244). It follows that at least one parent of the cross carried the genes *sc* and *sg*, and since it is known that the eyeless parent did not, it must be that the wild-type parent did. This parent therefore had the genotype *ey*$^+$ *sc sg* +, which gives normal growth rate since *sc* and *sg* are suppressed by *ey*$^+$.

Cross no. 2 and similar crosses made subsequently also gave rise to small-colony clones though neither parent showed this character. This means that the wild-type strain of *minus* mating-type carried the *sc* gene. Some slow-growing clones are expected in the progeny of cross no. 2 since the eyeless parent was itself slow-growing. If the *sg* gene were carried by the eyeless parent only, some recombinant clones of genotype *ey sc*$^+$ *sg*$^+$ with normal growth rate would be expected also, but no eyeless clones with normal growth rate have ever been recovered from crosses of this type. It seems highly probable therefore that the wild-type strain of *minus* mating-type carried the *sg* gene.

The hypothesis of a multiple mutation having occurred emerges from the observation that irradiation of a clone of genotype *ey*$^+$ *sc sg* (the wild-type *minus* strain) has led to the recovery of a clone with the genotype *ey sc*$^+$ *sg*$^+$ (the eyeless mutant). If mutations at these three loci are independent of each other the possibility of simultaneous mutation at all three is remote but not unreal. Alternatively one might postulate some mutational relationship between the three loci. This intentionally vague suggestion takes into account the possibility that the loci show similar instability towards a particular mutagenic agent. It also allows the existence of some mechanism whereby mutation at one locus is accompanied by or increases the likelihood of mutation at the other two. There is ample evidence from work on other organisms that mutations at different loci are not always independent of each other, though no case is known to the author which is closely comparable to that described here.

Frankel (1950) lists several instances where simultaneous mutation of both alleles at a locus has been reported, and himself describes a chlorophyll-deficient type of wheat which differs from both parents by genes at three loci. These loci show independent inheritance, but since wheat is hexaploid it is not unlikely that the three loci are homologous. The evidence is compatible with simultaneous mutation of both alleles at all three loci. The appearance in the mutant plants of a large inverted chromosome duplication is regarded by Frankel as more than a coincidence, and he interprets the structural change as a possible " destabiliser " of heredity. The work of McClintock (1951) on chromosome rearrangement in relation to mutability lends considerable support to this interpretation. It is not known if there is any structural change in the chromosomes of the eyeless mutant

of *C. reinhardi*. The small size of the cells makes it doubtful whether the chromosomes could be studied in detail.

Mampell (1945, 1946) presents evidence for a substance in *Drosophila* which causes mutability at a number of different loci in different chromosomes. The majority of genes affected by this mutator produce a minute phenotype when mutated, and may therefore be related in some way other than their positions on the chromosomes. In *D. pseudoobscura* formation of the mutator depends on a recessive gene and the presence of a Y-chromosome, but when transferred to *D. melanogaster* the substance is self-reproducing. It is conceivable that the three mutations believed to have occurred in *Chlamydomonas* were produced, not by the direct action of ultra-violet radiation on the loci concerned, but by a secondary mutagen, itself the product of ultra-violet radiation, to which all three loci are susceptible. The physiological relationship which has been demonstrated between these three loci may be of significance in this respect.

Tedin and Hagberg (1952) describe the results of treating seeds of *Lupinus luteus* with X-rays. One of the plants grown from these seeds was shown to carry three recessive mutant genes which, when homozygous, severally cause reduced chlorophyll content, lack of basal branches, and sterility. None of the genes was known to be present in the original material, and there is strong evidence that all three are on the same chromosome. The plants have a chromosome number of 52 so that while the chance of producing three independent mutations is itself very small, the chance that all three will be on the same chromosome is infinitesimal. It is concluded that the three mutations are not independent of each other and that linkage has made possible " some kind of chain reaction in one chromosome, or some other complicated phenomenon ". It is unlikely that a similar event has occurred in *Chlamydomonas* since there is evidence that neither *sc* nor *sg* is linked to *ey*, though in the absence of data to the contrary the possibility that they are linked to each other cannot be ruled out.

7. SUMMARY

Following irradiation of wild-type cells of *Chlamydomonas reinhardi* a mutant clone lacking the eyespot has been isolated. Presence or absence of the eyespot behaves as a single gene difference. Crosses between this mutant (*eyeless*) and the wild-type have revealed the existence of two genes affecting colony size, which are suppressed by the wild-type allele at the eyeless locus. The characters concerned are called *small-colony* and *slow-growth*. The wild-type strains carry both these genes but also carry the suppressor, so the characters are not expressed. After mutation to the eyeless condition, the original mutant clone no longer carried the genes for reduced colony size but alternative alleles giving normal colonies. It is deduced that mutation at the eyeless locus was accompanied by mutation at the other two loci.

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CYTOLOGICALLY ACTIVE SUBSTANCES PRODUCED BY SOIL MICRO-ORGANISMS

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SUBSTANCES showing activities which may be detected by cytological observations are nowadays known from many sources. Broadly speaking, cytological tests permit to discover at least two kinds of action: antimitotic, or altering the cell cycle, and mutagenic, or chromosome breaking. The two actions are not necessarily unrelated, and may be shown by the same substance, but it seems convenient to keep them distinct in a first simple classification.

While some of the substances known are synthetic in origin, and some are extracted from plants (more rarely from animals), a few new antimitotic drugs have been discovered which are produced by Actinomycetes (*e.g.* actinomycin, Hackmann, 1953). The discovery of mutagens of natural origin (d'Amato, 1950) makes it likely that among metabolic products of micro-organisms not only antimitotics, but also mutagens can be found. In view of these facts, a survey of cytologically active substances from microbial sources was undertaken. Soil micro-organisms, recently isolated, belonging to the classes of Schyzomycetes, Phycomycetes, Ascomycetes and Fungi Imperfecti were employed. Culture filtrates on suitable media were tested for cytological activity by means of the standard test on *Allium cepa* root tips.

1. MATERIAL AND METHOD

Micro-organisms were isolated from samples of soil collected in various places (mostly around Milan) by means of the usual plating procedures on agar media suitable for the isolation of bacteria, actinomycetes, fungi. The production of culture filtrates for use in cytological analysis demanded the choice of a suitable medium, as it was found that most standard media, both synthetic and not, are unfavourable for root growth and greatly decrease the frequency of cell divisions visible on microscopical examination. A medium was selected which, while permitting good growth of most of the strains to be examined, interfered least with the development of root tips, consisting of a carrot infusion broth supplemented with 2 per cent. glucose.

Seven-day-old aerated cultures were filtered, and young roots of onions exposed to the filtrates overnight. Root tips were stained with acetocarmine and squashed after heating to the first boiling.

2. EXPERIMENTAL RESULTS

The results of the cytological examination can be briefly related as follows. One type of alteration was fairly commonly observed in the treated roots, namely C-mitotic poisoning. With little variation, at least from a qualitative point of view, a similar picture emerged

with a variety of filtrates : metaphase blocks reminding more or less closely of those obtained by the action of colchicine. Quantitatively, of course, there was variation from filtrate to filtrate, and a few filtrates showed activities up to complete block of all mitoses observed.

More rarely were other kinds of cytological alterations noticed. Three filtrates showed signs of mutagenic action, chromosome fragments being observed with two filtrates, and chromatin bridges in another instance.

A summary of the activities detected is given in table 1, where the 102 filtrates tested are subdivided according to the main systematic groups. As will be seen from the totals, about 22 per cent. of the filtrates showed some degree of cytological activity, most of the cases (19 per cent. of the total) representing C-mitotic activity.

In almost all groups of micro-organisms tested was some activity found, except in the Phycomycetes, and there perhaps because of the small number examined. The distribution according to systematic

TABLE 1

Distribution of micro-organisms showing cytological activity according to main systematic groups. Figures between brackets refer to numbers of strains showing activity other than C-mitotic

Groups	No. active	No. inactive	Total
Eubacteriales	7 (2)	7	*14*
Actinomycetales	7	54	*61*
Fungi imperfecti	2	5	*7*
Phycomycetes	0	4	*4*
Ascomycetes	5	5	*10*
Non-identified fungi	2 (1)	4	*6*
Totals	**23**	**79**	**102**

groups shows some heterogeneity ($\chi^2 = 16{\cdot}35$ with 5 d.f.), the frequency of active filtrates being significantly smaller in the Actinomycetes. This conclusion is not, however, permissible at this stage, in view of the method of selection of micro-organisms employed. In fact, while for groups other than Actinomycetes, selection was practised in favour of micro-organisms endowed with antibiotic activity, Actinomycetes were sampled at random. The lower frequency of active filtrates in the latter group might therefore be the consequence of a correlation between antibiotic and antimitotic activity, such as would arise if the same substance were often endowed with both properties.

Antibiotic activity was tested by means of eleven indicator strains. A direct analysis of the correlation between it and cytological activity is given in tables 2, 3, of which neither shows significant correlation. On the other hand, these tables are not significantly at variance with the hypothesis that a low correlation exists, of insufficient magnitude to be detected by the use of unselected samples of the

present size, but capable of affecting the frequency of one type of activity in a significant way, when the other type of activity is selected.

TABLE 2

Correlation between antibiotic and antimitotic activity in Actinomycetes

		Antibiotic activity		Total
		+	−	
Antimitotic activity	+	2	11	*13*
	−	5	43	*48*
		7	**54**	**61**

TABLE 3

Correlation between antibiotic and antimitotic activities in micro-organisms other than Actinomycetes

		Antibiotic activity		Total
		+	−	
Antimitotic activity	+	9	4	*13*
	−	19	6	*25*
		28	**10**	**38**

TABLE 4

Correlation between antibiotic activity against single indicator strains and antimitotic activity

Indicator strain	Antimitotic activity	
	+	−
Brucella	3	6
Escherichia	6	3
Pasteurella	4	1
Proteus	3	3
Pseudomonas	2	2
Corynebacterium	6	11
Staphylococcus	6	9
Mycobacterium	1	4
Aspergillus	3	6
Candida	1	2
Torulopsis	0	6
	35	**53**

The problem of the correlation between antibiotic and antimitotic activity was tested further by analysis for the single indicator strains, as given in table 4. There is no significant heterogeneity ($\chi^2 = 7{\cdot}64$

with 10 d.f.) ; but, on grouping the indicator strains according to the simplest scheme, *i.e.* into Gram positives, Gram negatives, and others, some heterogeneity appears (table 5 ; $\chi^2 = 6{\cdot}20$ with 2 d.f., $P<5$ per cent.) pointing to a slight correlation between action against Gram negative micro-organisms, and in the *Allium* test. It is clear, however, that this point would need further investigation.

The active strains were classified into the following genera :

EUBACTERIALES : *Bacillus, Corynebacterium, Micrococcus.*

ACTINOMYCETALES : *Actinomyces* and *Streptomyces.*

MONILIALES : *Fusarium, Stysanus.*

ASCOMYCETES : *Aspergillus* (*glaucus, fumigatus*) ; *Penicillium* (*spinulosum*, and an unidentified *Penicillium* of the asymmetrical group).

Further tests were made to inquire whether the particular medium employed was responsible for the high frequency of antimitotics

TABLE 5

Correlation between antimitotic activity and antibiotic activity against certain groups of micro-organisms

	Antimitotic activity	
	+	−
Gram negatives . . .	18	15
Gram positives . . .	12	20
Others	5	18
Totals . . .	35	53

produced. Seven active strains were re-tested after growth on a synthetic medium plus yeast extract and none failed to reproduce the activity in the new conditions.

Finally, it was tested whether the C-mitotic action observed could lead to doubling of the chromosome complement of the cell. Onions, the roots of which had been exposed overnight to the action of filtrate 474 were placed again into tap water and examined after various times. Several polyploid mitoses were observed. It can therefore be asserted that some at least of the active principles can act as polyploidising agents.

3. DISCUSSION

The production of substances active on the cell cycle of higher organisms in cultures of micro-organisms isolated from the soil seems interesting under several points of view. Firstly, it is perhaps surprising to find that metabolic products of micro-organisms are so often endowed with selective toxicity for the reproduction processes of the

cells of higher organisms. It is of interest to compare their frequency (about 20 per cent.) with that with which antibiotic activity is detected (from unpublished data on 847 micro-organisms of origin and distribution comparable with that of the strains used in the present survey) :

Indicator strain	Frequency of antibiotically active soil micro-organisms
Staphylococcus aureus	10·7 per cent.
Corynebacterium diphtheriae	10·1 ,,
Pseudomonas pyocyanea	2·7 ,,
Proteus vulgaris	2·7 ,,
Escherichia coli	1·2 ,,
Mycobacterium minetti	0·5 ,,
Aspergillus niger	2·0 ,,
Candida albicans	0·4 ,,

These frequencies are so largely dependent upon the method used for the test that most conclusions based on them would seem unwarranted, but it seems at least permissible to conclude that antimitotic compounds are produced by micro-organisms at a rate similar, or higher than that of antibiotics.

A second consideration of general interest arising from the present research is that micro-organisms normally present in the soil are likely to be active determiners of polyploidisation in plants. It is obviously difficult to distinguish between " spontaneous " polyploidisation and that induced by agents of microbial origin, except by special experiments. But, at the present stage, one would see no reason why this particular kind of interaction should not take place in nature as well. The main limiting factor is, of course, the concentration of active substances that can be reached in the soil. The high numbers of germs found in the soil, and the frequency with which active producers of antimitotics are available, make it likely that sufficient concentrations are often attained.

The relative infrequency of mutagenic agents may also deserve some comment. Permeability conditions are, of course, to be kept in mind as a possible source of bias. Change of cultural conditions might also seriously affect the picture. Finally, the presence of natural antimutagens might obscure it.

A further point to be stressed is the desirability of using cytological tests in screening programmes, in the search of drugs active against diseases which, like cancer and leukemias, are the result for abnormal cell proliferation. There is already proof that drugs of this kind can be produced by micro-organisms. The frequency with which antimitotics can be found encourages us to believe that a wealth of new substances, some of which may be useful in human therapy, is thus made available. Cytological tests may prove very useful in this connection ; even if they are more laborious than screening methods for antibiotics, they are certainly far easier than methods using

experimental tumours. Should an antimitotic turn up to be antibiotic, the task of chemical isolation would certainly be simplified, but the chances for this seem to be fairly low.

4. CONCLUSIONS AND SUMMARY

Metabolic products of micro-organisms are found showing antimitotic activity with a rather high frequency. Active strains were obtained belonging to the Eubacteriales, Actinomycetales, Moniliales, and Ascomycetes, by means of a screening test using *Allium cepa* root tips. Mutagenic action was less frequent and striking. The correlation of antibiotic and antimitotic activity is not clear-cut and, if any, cannot be high. Antimitotic agents from soil micro-organisms are likely to act as powerful polyploidising agents in nature, probably explaining at least part of the "spontaneous" origin of polyploids. The advantages of cytological screening tests in the search for antimitotic drugs have been briefly discussed.

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POLYMORPHISM AND TAXONOMY

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This article is based upon an address delivered at Christchurch, New Zealand, on 17th May 1951, during the Seventh Science Congress of the Royal Society of New Zealand. It occupied pp. 245-253 of the published Report on that Congress,* which was published in January 1953. Apart from a few preliminary generalisations here omitted, it contains an account of some genetic work which had occupied a considerable amount of time for several years. The volume in which it appeared is by no means easily accessible outside the Dominion ; but it was thought that the offprints, which it was proposed to distribute rather widely among geneticists, would both provide easy access to the data here presented, and help to draw the attention of biologists to a Symposium which contains valuable contributions to many aspects of science. These offprints, however, were lost in transit from New Zealand to Britain. Accordingly, the Editors of *Heredity* were so good as to suggest that the article might be reprinted in this journal, if the necessary permission could be obtained. This was readily granted by the Royal Society of New Zealand, for which I am most grateful. I wish also to express my sincere thanks to the Editors of *Heredity* for insuring wider publicity to this work.

E. B. F.

POLYMORPHISM has been defined (Ford, 1937, 1945) as the occurrence together in the same habitat of two or more distinct forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation. The essential features of this definition have often been discussed (see also Ford, 1952), so that it is only necessary to point out here that it excludes continuous variation (as exemplified by human height), geographical races, the segregation of rare recessives (such as the majority of albinos), and the appearance of heterozygous mutants subject to elimination by selection. However, the term necessarily includes two distinct situations. A permanent or *Balanced Polymorphism* is due to a balance of selective agencies in which diversity is favoured and uniformity opposed : as in sex, or in all those instances in which the heterozygote is at an advantage compared with either of its corresponding homozygotes. On the other hand, a *Transient Polymorphism* involves only the temporary diversity of a population, which takes place while a previously disadvantageous gene spreads and displaces its allelomorph. This will generally occur owing to change in the environment or when a species colonises a new habitat.

Now a balanced polymorphism provides certain outstanding advantages for the study of evolution by experimental means. On

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the one hand, since discontinuous variation is involved, some simple switch-mechanism must control the development of one or another of the polymorphic forms ; and this is usually (though not always) achieved by the segregation of a pair, or at the most very few pairs, of genes. Moreover, the population is, as it were, " sensitised " to the occurrence of both the allelomorphs at such loci, since even the rarer of them will be much commoner than those that are maintained only by mutation pressure. Thus, in a balanced polymorphism there is ground for supposing that the adjustments of the gene-complex to a particular gene may often take place rather quickly and thus respond to the diverse conditions of different environments. It will be realised that continuous variations, controlled wholly on a multifactorial basis, may be expected to react even more quickly to selection ; yet the segregation of a pair of allelomorphs provides certain advantages for genetic analysis absent from the multifactorial situation.

Bearing these facts in mind, it seemed worth while to use balanced polymorphism to obtain some information on the genetics of geographical races. Thus, among other aspects of this question, it is desirable sometimes to analyse what is meant by the statement, frequently found in the literature of systematics, that the same form of a species occurs in two different parts of its range. In such a context " the same " usually means little more than visible or measurable similarity and we rarely have any indication whether or not the apparent identity extends to the mechanism producing it.

In Britain the moth *Triphaena comes* Hb. (family Agrotidæ) provides suitable material for such an enquiry. This species varies in size from about 4 to 4·5 cm. across the expanded wings. It has an extremely wide distribution and is generally common. In England and southern Scotland it is, with rare exceptions, monomorphic. The fore-wings are of a pale ochreous-brown, the hind-wings yellow with a black sub-marginal band.

In some parts of central and north Scotland and in the Scottish islands, however, this is a dimorphic insect, for a dark variety, *curtisii* Newmn., constitutes a considerable proportion of the population. Its frequency in different areas has not yet been established. It may, in some parts, reach 50 per cent. of the population, perhaps more ; though nowhere, to my knowledge, does it so far displace the normal *comes* that this is reduced to the status of a rare variety. *T. comes comes* is subject only to minor variation in colour, sometimes becoming rather greyish or reddish, but *T. c. curtisii* is extremely variable : the fore-wings range from a mahogany shade to intense black, while the yellow of the hind-wings is also clouded with black scales, slightly in the less dark forms but sometimes very heavily in those that are blackish. The correlation between the amount of melanin on the fore and hind wings is, however, far from complete, which much increases the difficulty of classifying the *curtisii* forms.

As long ago as 1905, it was shown by Bacot that *curtisii* is unifactorial

and nearly, but not quite, dominant. The heterozygotes are nearly always distinguishable from the recessives (the normal *comes*) without difficulty but heterozygous and homozygous *curtisii* overlap so that, when segregating, these two genotypes tend to fall in a bimodal distribution, or at least one in which the extreme dark forms are unduly common. Thus it seems that the darkest *curtisii* are nearly always homozygotes and the lightest heterozygotes, but those of intermediate shade cannot be assigned with confidence to one or the other class.

As is usual when all three genotypes are to any degree distinguishable, the heterozygotes are the most variable and the commoner homozygotes (*comes*) the least. These variations of *curtisii* are certainly largely genetic, for doubtless that form is sensitised to the effects of many other genes against which *comes* is buffered. It is unfortunate that the colour-phases occurring within the range of activity of the *curtisii* gene, both when heterozygous and homozygous, have received many distinct names (*rufo-nigrescens*, *nigrescens*, *nigra*, and others) which are often used in place of *curtisii* as if something distinct from it, so obscuring the genetic situation. In the present account these will be ignored, and *curtisii* extended to cover all the colour-phases controlled by the semi-dominant gene (*C*), responsible for the dark forms, compared with the pale recessive *comes* (*cc*).

It has long been known that *curtisii* occurs on the mainland of northern Scotland and that it is also found in the Hebrides on the west coast, and in Orkney at the eastern end of the north coast. The form is said to be similar in these two groups of islands and I could see no difference when specimens from them were compared. Yet Barra, in the Outer Hebrides, whence my western material was obtained, is in a direct line 100 miles from the nearest point on Orkney, though the Scottish mainland lies between them. However, Barra is separated by 25 miles of sea from the nearest point on the mainland, and by 15 miles from the island of Skye, which is so nearly a peninsula that it may be treated as a part of the Scottish coast. Between Orkney and the north coast of Scotland runs the Pentland Firth, six miles wide at the narrowest point, in which is the small wind-swept island of Stroma.

It is clear, therefore, that the population of *Triphaena comes* on Barra and Orkney are very isolated from one another. For this insect is not a regular migrant, a fact confirmed by the circumstances that specimens of the *curtisii* forms do not become scattered from northern and central Scotland, where they are common, to be caught as stragglers in the south of that country or in northern England. Similarly, a small and exceptional colony of dimorphic *T. comes* in which *curtisii* occupies a proportion of the population does, or did, exist on the sandhills of Lancashire. Yet *curtisii* was quite restricted to it.

I obtained specimens of *Triphaena comes* from Barra and Orkney in order to compare their genetics. The few females that reached

me (providing my first generation, B.I from Barra and O.I from Orkney) had all been fertilised in the open by unknown males. Four refused to lay, and I lost completely three broods of larvæ owing to disease. Unfortunately the species produces but one generation per year. However, the larvæ feed on many common low-growing plants and are easy to rear.

The insects obtained during the course of this work were classified into 5 colour-groups as shown, for each brood, in the table, in which

Table of broods

Brood	v. dark *curtisii*	dark *curtisii*	*curtisii*	*curtisii* to *comes*	*comes*	Total
B.I.3	...	...	1	...	...	1
B.I.5	...	1	...	...	...	1
O.I.1	...	1	...	...	...	1
O.I.2	...	1	...	...	...	1
O.I.7	...	1	...	...	...	1
B.II.3	1	6	21	...	15	43
B.II.4	8	6	25	1	10	50
O.II.1	...	14	29	...	36	79
O.II.2	...	3	3	...	...	6
O.II.3	...	1	...	...	1	2
B.III.1	...	10	31	...	...	41
O.III.1	...	9	18	...	39	66
O.III.2	1	25	41	...	58	125
B.IV.1	29	23	54	2	27	135
B.IV.2	2	...	4	...	1	7
B.IV.3	9	6	22	1	14	52
B.IV.5	...	...	...	...	2	2
O.IV.1	31	18	31	2	24	106
O.IV.2	5	8	17	...	5	35
B.O.IV.2	10	14	24	11	15	74
B.O.IV.4	3	4	7	4	5	23
B.O.IV.5	11	10	16	7	19	63
B.O.V.2	21	14	...	...	...	35
B.O.VI.1	13	1	...	...	...	14
B.O.VI.4	10	27	...	...	...	37
B.O.VI.5	68	9	1	...	...	78

the full data are recorded. The three darkest of these represent *curtisii* forms (very dark, dark, and normal *curtisii*). Then follows the one ("*curtisii* to *comes*") into which insects approximately intermediate between *curtisii* and *comes* were placed, while the lightest specimens represent *comes* itself. Owing to the lack of close correlation between the amount of darkening on the fore and hind wings and the mottled appearance of some of the specimens, it was often difficult to decide

where the more intermediate *curtisii* should be included; consequently the " dark *curtisii* " group is rather arbitrary. The distinction between *curtisii* and *comes* was, however, nearly always clear, as was the correct classification of the darkest and lightest *curtisii*.

From the Barra insects I succeeded only in rearing two broods which constituted my second generation (the first reared in captivity). One of these, B.II.3, from the *curtisii* female B.I.3, proved rather anomalous, giving 28 *curtisii* forms (18 ♂, 10 ♀) and 15 *comes* (6 ♂, 9 ♀). It would indeed have been worth while to explore this situation, but the work would have required more time and space than I could afford.

My other brood from Barra, B.II.4, from the dark *curtisii* B.I.5, produced 39 *curtisii* forms (22 ♂, 17 ♀), 10 *comes* (7 ♂, 3 ♀) and a single male of intermediate shade. This is clearly an F.2 segregation, as indicated also by a group of extreme black insects certainly representing some of the homozygotes.

I raised three broods from wild Orkney females, which were all dark *curtisii* (O.I. 1, 2, 7), but two were of negligible size. They were as follows: O.II.1, 43 *curtisii* forms (17 ♂, 26 ♀); 36 *comes* (17 ♂, 19 ♀), evidently a 1 : 1 segregation; O.II.2, 6 *curtisii* (4 ♂, 2 ♀); O.II.3, 1 *curtisii* and 1 *comes* (both ♂).

I feel some uncertainty as to whether brood B.II.3 represents a disturbed 3 : 1 or 1 : 1 ratio. B.II.4 is evidently segregating as a result of a mating between 2 heterozygotes, while O.II.1 is clearly a backcross family. O.II.2 and 3 are too small to distinguish between the two types of ratio.

It was now necessary to produce parallel generations (Gen. III) which would, with certainty, provide me with known heterozygotes. The insects emerged over a long period, making it difficult to obtain the requisite pairings. However, in the Barra line, using family B.II.4, I crossed two of the very black male *curtisii* with female *comes* but successfully reared only one brood (B.III.1) of 41 insects (16 ♂, 25 ♀), all *curtisii* and none of the darkest shade. These evidently were heterozygotes.

In the Orkney line I had to make backcrosses using individuals of family O.II.1. These provided me with two families in the succeeding generation (that parallel to B.III.1). They consisted of O.III.1, 27 *curtisii* forms (14 ♂, 13 ♀); 39 *comes* (24 ♂, 15 ♀); and O.III.2, 67 *curtisii* (26 ♂, 41 ♀), and 58 *comes* (32 ♂, 26 ♀). Here evidently, as expected from the parents, the total of 94 *curtisii* were also all heterozygotes. The extreme black forms were absent from them, as in B.III.1, except for a single specimen in O.III.2 which can hardly be other than an abnormally dark heterozygote.

We now reach the critical generation of the experiment. In the Barra line a number of pairings were obtained between males and females of heterozygous *curtisii* in brood B.III.1, and two large broods, each segregating in a 3 : 1 ratio resulted from them. They were

B.IV.1, 106 *curtisii* (46 ♂, 60 ♀), 27 *comes* (10 ♂, 17 ♀) and 2 intermediate specimens (1 ♂, 1 ♀). Also B.IV.3, 37 *curtisii* forms (15 ♂, 22 ♀), 14 *comes* (5 ♂, 9♀) and 1 intermediate male. There were also two families which were nearly lost through disease : B.IV.2, 6 *curtisii* (all male) and 1 *comes* (female) ; also B.IV.5, 2 *comes* (1 ♂, 1 ♀).

In the Orkney line, 2 heterozygous *curtisii* in O.III.1 were mated to give two broods : O.IV.1, 80 *curtisii* (49 ♂, 31 ♀) and 24 *comes* (15 ♂, 9 ♀), with two doubtful specimens both male. Also brood O.IV.2 of 30 *curtisii* (11 ♂, 19 ♀) and 5 *comes* (all male).

While the brood III insects were emerging, it became clear that the time had arrived to allow the *curtisii* gene to segregate as an F.2 ratio in a mixed Barra and Orkney genetic background. For it was by then fairly evident that the *comes* form is almost fully recessive. Indeed, when the whole of the third generation broods could be examined, it was seen that among a total of 412 segregating specimens (252 *curtisii* ; 159 *comes*) in the second and third generations, only one was doubtful. The B.I and O.I insects are here excluded as they might have been selected. These numbers were later raised with the addition of the B.IV and O.IV broods, to 6 unclassifiable specimens (those listed in the table as " *curtisii* to *comes* ") out of 749 (comprising 511 *curtisii* forms and 232 *comes*).

Accordingly a (heterozygous) male from B.III.1 was crossed with a heterozygous Orkney female from O.III.1 to give the brood B.O.IV.2 of 74 insects (43 ♂, 31 ♀), and brood B.O.IV.4 of 23 insects (9 ♂, 14 ♀), resulting from a similar pairing. Also, making the reverse cross, a heterozygous female from B.III.1 was mated with a heterozygous male from O.III.2, producing B.O.IV.5 of 63 insects (38 ♂, 25 ♀). Several more of these crosses were tried but were failures.

In broods B.O.IV.2, 4 and 5, segregation in the ratio 1 *CC* : 2 *Cc* : 1 *cc* was, of course, taking place, but it did not produce a clear discontinuity between *curtisii* and *comes* as heretofore. On the contrary, a failure of dominance occurred, and the insects consisted of an almost unclassifiable series from normal *comes* to the darkest *curtisii*. It is especially to be noticed that this was confirmed in all three broods in which the genetic background of the Barra and Orkney insects had been mixed.

One point which gave rise to a good deal of trouble remains to be considered. It might be suggested that *curtisii* in Barra and Orkney is produced by different genes having similar effects (like the three genes for Rex coat in the rabbit, or four of the genes producing *retinitis pigmentosa* in Man). The interaction which might then occur on crossing could result in a seriation which it would be difficult to unravel, and similar in general appearance to that encountered.

It is easy to test the identity of two mutant genes when recessive in effect, but more difficult to do so when they are semi-dominants. In this instance a mating was obtained between a pair of the blackest available specimens respectively of Barra (the male) from B.IV.1

and of Orkney origin (the female) from O.IV.1. A family of 35 specimens (22 ♂, 13 ♀), constituting B.O.V.2 resulted from this and all of them were of the darker *curtisii* forms. Three successful broods were produced by inter-breeding insects of this family: B.O.VI.1 of 14 (10 ♂, 4 ♀), B.O.VI.4, of 37 (14 ♂, 23 ♀) and B.O.VI.5, of 78 (46 ♂, 32 ♀). The whole of these 129 insects comprising the B.O.VI broods were *curtisii* and all but one were of the darker forms.

This evidence requires a brief examination. Suppose that two genes are in fact involved: true *curtisii* (*C*) and a visibly similar but genetically distinct one for dark colouration (*D*). By crossing homozygous dark insects from the two lines respectively (B.IV.1 and O.IV.1), each would then bring in the normal allelomorph of the other, and the brood B.O.V.2 would have the genetic constitution *CcDd*. If the two genes were additive in effect, the same result as that produced by *CC* would be obtained. If they were not additive, either the paler shade of the heterozygotes or some new colouring would be produced. These latter alternatives are excluded by the results.

However, on inter-breeding insects of B.O.V.2 if of the constitution *CcDd*, segregation should occur in a ratio 9 : 3 : 3 : 1 and one insect in 16 would be normal *curtisii*. The 129 specimens, all dark, that were obtained are sufficient to exclude this.

It may be objected, however, that in choosing the specimens from broods B.IV.1 and O.IV.1 homozygotes were not correctly identified and selected for breeding, and that one of these parents (clearly not both) was heterozygous. This would produce in B.O.V.2 a segregating brood of the hypothetical constitution 1 *CcDd* : 1 *Ccdd* (or the reverse). Such a misclassification, therefore, could only produce some light specimens, and in addition, raise the chances of obtaining normal *comes* in the succeeding (B.O.VI) generation. Thus it appears that the *curtisii* form in Barra and Orkney is due to the same gene (or to an allelomorph of it).

I. DISCUSSION

The *curtisii* and *comes* forms prove to be clearly distinguishable, save on rare occasions, in the bred lines derived from Barra (Outer Hebrides) and Orkney females. Thus, among the 749 insects obtained in segregating families, I was only in serious doubt about the classification of about six specimens. However, there was a break-down in dominance when the two gene-complexes were mixed by inter-breeding, for the two classes were then no longer distinct (broods B.O.IV.2, 4 and 5). That is to say, since the *curtisii* gene was proved identical in these populations, dominance must have been achieved in them by the selection of a different set of " modifiers ".

The experimental design necessary to establish these results differs somewhat from that employed in other studies on dominance-modification. Thus, in his celebrated work on poultry, Fisher (1935, 1938) was concerned to put certain genes characteristic of some domestic

breeds back into the genetic setting of the wild jungle-fowl in which they must normally have the status of rare mutants. For that purpose it was necessary to make repeated back-crossings to the wild birds until the gene to be studied was immersed in a gene-complex which was predominantly that of the jungle-fowl type. Yet such repeated back-crossings would presumably have tended to restore dominance after inter-crossing the Barra and Orkney lines of *Triphaena comes*: for here, unlike the poultry instance, we are dealing with two races in both of which the same gene is common. By taking *curtisii* genes of Orkney origin and placing them in the genetic environment of the Barra insects, it may be presumed that *curtisii* would merely re-acquire semi-dominance in a somewhat different way, that appropriate to Barra. And, of course, a comparable result would be produced by the reverse procedure.

In these instances, the maximum lack of balance was obtained from the cross between Barra and Orkney material, without subsequent back-crossing. It was not deemed necessary to explore this situation by inter-breeding these families of mixed origin (B.O.IV.2, 4 and 5). Not because this would have been unrewarding, but because a breakdown sufficient to illuminate the taxonomic situation had been obtained without doing so, presumably because many of the genes by which the gene-complex is adjusted to give dominance were themselves operating on an additive and multifactorial basis. Moreover, such work requires so much time and space that nothing more could be undertaken than was requisite for the enquiry on hand.

It is worth adding that a study of specimens in collections leads me to suspect that while *curtisii* and *comes* in general remain quite distinct on the Scottish mainland, they may there be more often connected by intermediates which it is hard to classify than in the material from Barra and Orkney. It is, indeed, not unexpected that the greater isolation of an island may promote a better adjustment to the *curtisii* gene. It is possible, therefore, that when *curtisii* is bred from mainland localities, especially from the southern part of its range, the segregation, though still clear, may be less sharp than I myself found.

We cannot, of course, suppose that semi-dominance has been attained independently from the intermediate heterozygote in Barra and Orkney. For on the few occasions on which *curtisii* has already been bred, it proved to be semi-dominant also on the Scottish mainland, which intervenes between those islands (though it is hard to determine what the exact frequency of the intermediates may have been in such results). We may reasonably assume that the number of genes influencing dominance is very large, and that they will have other effects also. Where isolation permits it, selection will therefore act upon these to adjust them as may be required by the environment, while at the same time maintaining the balance necessary to ensure the semi-dominance of *curtisii*. Indeed, though an actual break in

distribution provides the most complete form of isolation, it does not provide the only geographical one. Mere distance can impede the free flow of genes and so build up a different adjustment, which may amount to sub-speciation, even at the opposite ends of a continuous cline (Ford, 1949).

It is now possible, therefore, in this instance, to extend the systematist's statement that the *curtisii* form of *T. comes* occurs both in the Outer Hebrides (Barra) and in Orkney, and to attach a genetic meaning to it. In both places *curtisii* is unifactorial, nearly dominant, and due to the same gene. But the similar adjustments of these two populations to that gene, giving it semi-dominance, have been achieved by different means : the " dominance modifiers " employed in Barra and Orkney are not the same.

In every fauna and flora, but particularly such as that of New Zealand, there are likely to be instances in which " the same " form occurs in distinct areas, and indeed, this situation will increase as the wild countryside becomes more subdivided by cultivation or the planting of imported timber. It would be a matter of much interest to determine the genetic basis of these, and other taxonomic situations, along the lines illustrated by the present work, in which advantage has been taken of the special properties of polymorphic material.

2. SUMMARY

1. The moth *Triphaena comes* (Agrotidæ) is monomorphic in England but dimorphic in central and northern Scotland and the Scottish Islands where, in addition to the normal *comes*, a dark form *curtisii* occupies a considerable proportion of the population. Its frequency in these localities has not yet been determined, but it is known to be high enough to indicate a balanced polymorphism.

2. The *curtisii* form occurs both in Barra (and elsewhere) in the Outer Hebrides and in the Orkney Islands, and these two populations are very isolated from one another.

3. *Curtisii* is unifactorial and nearly, but not quite, dominant to *comes*.

4. It has been shown that the same gene, or allelomorphs of it, control the *curtisii* forms in Barra and Orkney.

5. Though *curtisii* is apparently identical both in appearance and in genetic behaviour in these two places, it achieves that identity by different means, for the sets of " modifiers " which adjust its dominance are significantly different in these two islands.

6. Owing to the use of polymorphic material it has, therefore, been possible in this instance to analyse in some detail what is meant by the statement that " the same " form of a species occurs in two isolated localities.

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ECOLOGICAL GENETICS OF *MANIOLA JURTINA* L. ON THE ISLES OF SCILLY

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We have already shown that the distribution of spots on the under-side of the hind-wings of the butterfly *Maniola jurtina* provides a reliable means of quantifying variation in both sexes, and that it can be used as an index of evolutionary divergence in different colonies (1 ; 2 ; 3). In the male, spotting proved to be uniform throughout the islands studied and similar to that of the mainland populations. Spot-distribution in the females, however, was very variable and quite distinct from that found in the rest of England. While the three large islands (St Mary's, Tresco and St Martin's) all showed similar values, a number of smaller ones (the areas of which are one-seventeenth or less those of the large), exhibited a great diversity of spotting, each being characterised by a particular type of spot-distribution (3).

One of the main objects of our work in the Scillies during August 1953 was to discover whether the characteristics of the various island populations had changed appreciably during the two years which had elapsed since our previous visit.

1. THE SMALL ISLANDS

(*a*) *Females*

Samples of the female populations on a number of the small islands taken in 1951 and 1953 are compared in table 1.

It will be seen that, with one exception (Tean area 5, see map), there is no significant difference in spotting between the two years. In other words, spot-distribution on these small islands must have been stabilised during the period between successive samples. For Tean area 1 our information is more extensive and dates back to 1946. A table of general contingency for samples of females taken in 1946, 1950, 1951 and 1953 gives $\chi^2_{(9)} = 7{\cdot}2$ for which $P = 0{\cdot}7$ to $0{\cdot}5$. There is thus good reason for assuming that the population has remained stable in respect of spotting for a period of eight years.

The situation on Tean area 5 is of interest. Our collecting in 1951 had shown for the first time that a significant difference existed between the populations of areas 1 and 5, which had been similar in 1950 (3). Clearly, some effective ecological barrier now existed between them. This was undoubtedly area 4, a tongue of windswept

ground about 120 yards long and 60 yards wide with short, close turf and stunted plants such as *Lotus corniculatus*. Careful observation had shown that although the butterflies frequently set out from both

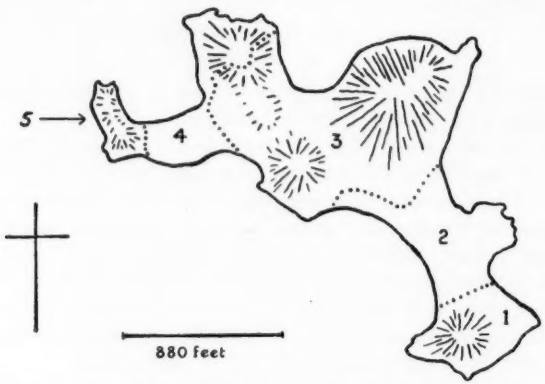
TABLE 1

Spot-distribution in female Maniola jurtina *on the small islands in 1951 and 1953*

	Spots						Total	Difference
	0	1	2	3	4	5		
St Helens (1951) .	26	41	64	18	3	1	153	N.S. ($\chi^2_{(3)} = 5{\cdot}80$; P = 0·1 to 0·2)
„ (1953) .	46	53	65	14	2	0	180	
Tean Area 5 (1951) .	49	12	42	3	4	1	111	S. ($\chi^2_{(2)} = 8{\cdot}02$; P = 0·02)
„ 5 (1953) .	30	14	54	13	3	0	114	
Tean Area 1 (1951) .	33	9	40	13	7	1	103	N.S.
„ 1 (1953) .	32	11	46	21	3	1	114	
Arthur (1951) . .	8	5	36	11	4	0	64	N.S.
„ (1953) . .	5	5	44	18	4	1	77	
Gt. Ganilly (1951) .	41	7	37	8	5	0	98	N.S. ($\chi^2_{(2)} = 3{\cdot}58$; P = 0·1 to 0·2)
„ (1953) .	32	13	52	6	2	0	105	

In the column for Difference, N.S. denotes " Not Significant " and S. denotes " Significant ". Figures for χ^2 are included where there is any question of doubt.

ends as if to cross this area, they always turned back after flying about 10 yards from the extremities. We never saw a *jurtina* in the middle of area 4. These observations were confirmed by marking



MAP.—Tean (Isles of Scilly). Boundaries of collecting areas . . . . . .

individuals in areas 3 and 5 with distinctive colours. No interchange was detected in the relatively large samples subsequently caught (including almost the whole of the population in area 5).

On revisiting Tean in 1953, it was at once obvious that the grass in area 4 was longer than ever before and was now at least six inches high. The reasons for this change were not far to seek. For some years the island had been continuously grazed by a herd of cattle, but these were removed in the late summer of 1950 and not replaced. Their absence made little difference to the vegetation during 1951 but by 1953 its increase was most striking. *M. jurtina* of both sexes could now be seen flying across area 4 in either direction, obviously gaining adequate shelter from the wind. It seems clear that the changed ecological conditions had been responsible for the alteration in spotting during the two intervening years, and it is not surprising to find that the populations in areas 1 and 5 are now identical (see table 1). The difference between them was measured by $\chi^2_{(3)} = 9{\cdot}2$ in 1951, and by $\chi^2_{(3)} = 3{\cdot}00$ in 1953.

(*b*) *Males*

The only sample collected on a small island was from Tean area 1 (see table 2).

TABLE 2

Spot-distribution in male Maniola jurtina *on a small island in 1951 and 1953*

	Spots						Total	Difference
	0	1	2	3	4	5		
Tean Area 1 (1951) .	1	5	50	15	9	3	83	N.S.
„ 1 (1953) .	2	8	73	21	9	1	114	

Clearly, the two are similar in spot-distribution. Owing to shortage of time, due to other work on which we were engaged, we thought it worth while only to obtain this one sample taken on the island where we camped. For we had already found (3) that the male spot-distribution is the same on all the islands mentioned in this section, large and small, and that this accords with its frequency-distribution throughout southern England. Thus we felt that its lack of variation from one locality to another, which contrasts so strikingly with the female situation, justified us in omitting further male counts this year, except for occasional checks such as the one included here and the samples taken on St Martin's (p. 268) and on Samson (pp. 270-1).

2. THE LARGE ISLANDS

(*a*) *Females*

Sampling in 1953 was confined to St Martin's (see table 3). Evidently there is no indication of divergence between the two years. Furthermore, a table of general contingency for samples in 1950,

1951 and 1953 gives $\chi^2_{(4)} = 1{\cdot}9$; $P = 0{\cdot}8$ to $0{\cdot}7$. Hence we are justified in assuming stability in the female population on the central area of St Martin's over a period of four years. Collecting in 1950 also established a similarity in female spotting between St Martin's and Tresco which remained unaltered in 1951, when it was also found to be similar to that on St Mary's (2; 3). Lack of time prevented us from obtaining samples on Tresco and St Mary's in 1953.

TABLE 3

Spot-distribution in female Maniola jurtina *on a large island in 1951 and 1953*

	Spots						Total	Difference
	0	1	2	3	4	5		
St Martin's (1951) .	38	38	33	5	2	0	116	N.S. ($\chi^2_{(2)} = 0{\cdot}92$; $P = 0{\cdot}7$ to $0{\cdot}5$)
„ (1953) .	46	34	36	3	1	0	120	

(*b*) *Males*

We took a small sample of males from St Martin's in 1953. The spot-distribution proved to be quite typical and served to confirm our view that male spotting is stabilised on all the islands we have visited so far, both large and small (see table 4).

TABLE 4

	Spots						Total	Difference
	0	1	2	3	4	5		
St Martin's (1951) .	8	18	199	43	18	7	293	N.S. ($\chi^2_{(2)} = 0{\cdot}15$; $P > 0{\cdot}9$)
„ (1953) .	...	7	37	9	3	...	56	

3. ISLANDS OF INTERMEDIATE SIZE

It has already been explained that the Isles of Scilly on which we have worked can be classified into large islands, of which there are three (St Martin's, Tresco, St Mary's), with areas of 682 acres or more, and into four small islands with areas of 40 acres or less. The smallest of the large islands (St Martin's) therefore has an area approximately seventeen times greater than the largest of the small (St Helens). The *M. jurtina* populations are adjusted entirely differently to the conditions obtaining on islands of these two size-groups.

Between them, however, are two, Bryher (353 acres) and Samson (80 acres), of intermediate area. It was a matter of much interest, therefore, to determine whether the female spot-distributions of the butterflies inhabiting them conform with the large-island or the small-island types, or are of a different nature from either.

(*a*) *Bryher*

The northern and southern parts of this island are high, bare, and rocky, and are unsuited to maintaining a *jurtina* population; the northern end, indeed, consists of a wind-swept heather moor. In the centre, where the village is situated, the ground is low-lying, but it is cultivated; so that there the butterfly maintains itself principally along the edges of fields and the borders of lanes. Consequently, it is uncommon, and the region acts as a partial barrier to the insect. It is, indeed, only along the lower slopes surrounding the southern hill, and the western and eastern sides of the northern one, that it flourishes.

We had sampled one of these communities, that on the east side of the southern hill, in 1951 (3) with the surprising result shown in table 5: that is to say, the distribution of female spotting descends from a single large mode at 0 spots, like one half of the normal male distribution, in the same way that it does on the mainland of England eastwards from the border of Cornwall and Devon (2, 3). Thus it is entirely distinct from the distribution on the three large islands in Scilly, which all have approximately equal frequencies at 0, 1, and 2 spots. In 1953 we sampled this same population on Bryher again (table 5) and found it to be substantially unchanged ($\chi^2_{(2)} = 2{\cdot}02$, for which $P > 0{\cdot}3$), though it is unlike any other known in Scilly.

Lack of time had in 1951 prevented us from examining the ecology of Bryher with respect to *M. jurtina* or sampling a further population there. These defects we remedied in 1953, with the result in regard to habitats already outlined. We captured 106 females from a considerable colony of the butterfly on the west side of the northern plateau, collecting on and around the isolated eminence of Gweal Hill: the data thus obtained are given in table 5. Here, as will be seen, the spot-distribution is unlike that at the southern end of the island, having a single mode at 2 spots. The difference between the two populations, of course, using the 1953 samples, is significant ($\chi^2_{(2)} = 7{\cdot}27$, for which $P < 0{\cdot}02$). These results suggest that *M. jurtina* on Bryher is split into distinct populations each of them equivalent, in extent and uniformity of environment, to those on two small islands.

That is to say, the insect does not here inhabit an extensive and heterogeneous area, as it does on the large islands. Accordingly, it has become adapted differently in the rather isolated localities in which we found it, for ecologically these are not like one another. That at the southern end consists of little fields of rather long grass

sheltered by stone walls, and of deep grassy tracks and tiny clearings among tall bramble and bracken. The other, on and around Gweal Hill, is much more exposed, with relatively large areas of grass, larger fields, and much less bracken and bramble.

(*b*) *Samson*

This island of 80 acres is effectively in two parts. Each consists of an oblong conical hill running roughly north and south, the sides of which are covered with tall bracken. Between them is a low flat isthmus of short turf, without bracken, and 75 yards wide. We found a small *M. jurtina* population on steep grassy slopes, relatively free from bracken, at the northern end of the island; and a second among and around the ruins of the deserted village on the summit

TABLE 5

Spot-distribution of female Maniola jurtina *on two islands of intermediate size, Bryher and Samson*

	Spots						Totals
	0	1	2	3	4	5	
Bryher, South, 1951 .	51	34	21	2	2	0	110
„ 1953 .	42	31	27	6	0	0	106
Bryher, North, 1953 .	26	33	46	3	1	0	109
Samson, North, 1953	9	7	39	10	1	0	66
Samson, South, 1953	10	12	38	1	1	0	62

of the southern hill, below which we also took a few along the narrow track between the bracken and the shore on the west side.

The elongated summit of the northern hill, on which are situated magnificent Chamber Tombs of the Bronze Age, is covered with short turf; so is the isthmus between the two parts of the island. This turf is quite unsuitable as a habitat for *M. jurtina*, which is rare or absent in both areas. Indeed, it is of the kind which forms an effective barrier to the insect on Tean.

On Samson, therefore, are two populations of this butterfly, one northern and one southern. They are isolated from one another yet identical (table 5), as formerly were the isolated populations in areas 1 and 3 on Tean (1, 2). It will be obvious that they are of the type in which the female spot-distribution is rather similar to the male, with a single large and clearly marked mode at 2 spots. They must be adjusted to effectively similar conditions, in so far as the multifactorial spot-distribution is an index of such adjustment; and, indeed, the ecology of their habitats does seem to be very much the same.

Here, as on Bryher, we have not one population of intermediate size between those on the large and small islands, but two small populations which are quite unlike the type with effectively similar frequencies at 0, 1 and 2 spots which seems to arise as a compromise to a wide diversity of conditions within a large habitat. Incidentally, it is worth noting that the nearest population to those on Samson is that at the southern end of Bryher, 750 yards across the sea at high water, and it is very different from them.

We obtained small samples of males from both the northern and southern populations on Bryher, and find that they are quite characteristic of the normal condition in *M. jurtina*. That is to say, they have a single mode at 2 spots (table 6) which, being the type found throughout nearly the whole range of the butterfly (3), does not reflect adjustment to different ecological conditions in the way that is indicated so clearly by female spotting.

TABLE 6

Spot-distribution of male Maniola jurtina *in two populations on the island of Samson in 1953*

	Spots						Totals
	0	1	2	3	4	5	
Samson, North .	0	1	29	14	7	0	51
Samson, South .	0	4	40	19	4	0	67

4. SUMMARY

1. The female spot-distribution of the restricted populations of *Maniola jurtina* on the small islands in Scilly are, in general, different from one another, but remain constant on each.

2. A single and distinct type of spot-distribution is, however, characteristic of the three large islands of St Martin's, Tresco, and St Mary's. This also remains constant from year to year. It seems to be developed in response to the average of the conditions existing in a considerable and diversified habitat.

3. The small island of Tean now contains two (formerly three) isolated populations. The main one had remained bimodal (with a smaller mode of no spots, and a larger at two) since 1946. This condition persisted also in 1953.

4. In 1951, the very small isolated population on area 5 of Tean had become significantly distinct, being bimodal but with the larger mode at 0. By 1953 this difference had disappeared. Moreover, we found area 5 no longer isolated from the rest of the island, for the lawn-like turf, unsuited to the butterfly, in the intervening region had grown long, owing to the removal of the cattle which had formerly grazed it.

5. It was found that two islands of intermediate size (Bryher and Samson) each contained two populations isolated from one another, and occupying limited areas. They, therefore, had the characteristics of those found on the small islands.

6. Thus the female spot-distribution of *Maniola jurtina* in the Isles of Scilly is maintained at a number of distinct and constant values by selection in response to local conditions. When the conditions change, however, so may the spot-distribution if the area affected be of small size.

5. REFERENCES

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REVIEWS

GENETIC HOMEOSTASIS. By I. M. Lerner. Edinburgh : Oliver and Boyd. 1954. Pp. 134. 12s. 6d.

Dr Lerner is well known for his earlier work *Population Genetics and Animal Improvement*, and his new book should therefore be assured of a wide and appreciative circle of readers. It deals with the same general topic of the genetical properties of populations, but differs from its predecessor in being less a wide survey of methods and problems than an essay on a particular and very important aspect of the subject. Populations are resistant to change in their genetical constitutions—they show an inertia, an element of self-regulation, or genetic homeostasis : Dr Lerner sets out to assess the evidence as to the nature of this resistance and to discuss its causes. In particular he seeks to relate it to the regulation of development, the canalisation, stability or developmental homeostasis, which is displayed by the individual organism.

The collection and appraisal of the evidence is thorough and most illuminating, the discussion of phenodeviants being especially valuable. No doubt can be left of the occurrence of both developmental homeostasis and genetical homeostasis (to use Lerner's term) or of their dependence on the balance and organisation of polygenic systems. Two of the conclusions that Lerner reaches will, however, be less widely acceptable. The first of these is that heterozygosity *per se* can be of importance in development, or to put it the other way round, " Not only gene contents but homozygosity as such must be considered to play a rôle in inbreeding degeneration " (p. 75). The second is set out on p. 118 in the words " The thesis here advanced is that the former (adaptedness of the individual) is mediated by heterozygous advantage in buffering ability as a consequence of which populations become endowed with the latter (adaptability). The buffering of populations against environmental change . . . is a by-product of the buffering of individuals ", and further (p. 120), " Error is minimised in a successful population by developmental homeostasis. Genetic homeostasis arises as an after-effect ".

If I read him aright, Lerner's view could be paraphrased in the following way. Heterozygotes, because they are heterozygous, show a better buffered development (*i.e.* show developmental homeostasis) and therefore have an advantage in fitness over homozygotes. Having such an advantage, they are held in the population, which will therefore conserve its variability and resist change under any force of selection favouring more extreme types and hence tending to increase homozygosity (*i.e.* show genetic homeostasis).

Now, few will seek to dispute that heterozygotes in general show more stable development (or better buffered development, or superior developmental homeostasis, or whatever form of expression one chooses) than do homozygotes in outbreeding species ; or that populations of such species resist change under selection (or show genetic inertia, or genetic homeostasis). To interpret this as depending on innate capacity of heterozygosity to give superior development is, however, another matter. There are

several objections to this view, which to me hedges a mechanical concept essential to the understanding of genic transmission with unproven physiological restrictions; but one of them must surely be decisive in itself. Heterozygotes do not show this same property in inbreeding species. Lerner seeks to meet this objection early in his book when he writes " . . . the hypothesis . . . is applicable only to populations with genetic systems based . . . on cross-fertilisation ". (p. 7). Inbreeders, apomicts and so on, do certainly differ from outbreeding species but hardly in the basic genetic principles upon which they depend. To argue to the contrary is to see rye resembling, for example, *Drosophila*, but differing in the basic properties of heterozygosity from wheat, with which it is so closely related as to give hybrids; or to endow heterozygosity in the tomato, as we know it in this country, with properties unknown in its South-American ancestor and yet shared by *Galeopsis* and barley. Inbreeding and outbreeding are too closely interwoven in evolution to allow of this escape.

Yet object as we may to Lerner's final conclusions, we cannot fail to thank him for his book. It not only sets facts before us but it makes us think about them, and it does so with a style of scholarly presentation which is all too rare. *Genetic Homeostasis* may be criticised, but it will not be ignored. No geneticist should fail to read it: we all have much to obtain from Dr Lerner. KENNETH MATHER.

EVOLUTION AS A PROCESS. Ed. Julian Huxley, A. C. Hardy, and E. B. Ford. *Contributors*: G. R. de Beer, E. J. H. Corner, H. B. Cott, James Fisher, Sir Ronald Fisher, E. B. Ford, J. B. S. Haldane, A. C. Hardy, Julian Huxley, David Lack, Ernst Mayr, Bernhard Rensch, P. M. Sheppard, H. N. Southern, N. Tinbergen, T. S. Westoll, E. N. Willmer, J. Z. Young and S. Zuckerman. London: George Allen and Unwin. Pp. 367. 25s.

At some stage in the preparation of this volume, its contributors hit upon the happy idea of turning it into an unpremeditated *Festschrift* in honour of its senior editor, Dr J. S. Huxley; and if all else in the book were controversial, the truth of its foreword, which speaks of Huxley's own splendid contributions to biology and the contributions which he has caused others to make, is surely not to be denied.

Huxley's own article, like Fisher's later on, deals with certain popular misconceptions about the content and import of the genetical theory of evolution by selection. Misconceptions there must be, in a world in which we cannot all be geneticists; but why are they so distressingly popular? How did it get about, for example, that evolution is the outcome of the blind selection of chance mutations (for that is what Darwinism is popularly supposed to be)? Perhaps geneticists themselves are partly to blame. Huxley writes:

> " Natural selection has certain obvious limitations. It can only produce results which are of immediate biological utility to the species; and being blind and automatic, it is incapable of purposeful design or foresighted planning."

I should be sorry to be obliged to defend this view against the censure of a determined critic. What can " blind " mean except undiscriminating and undirective, exactly that which selection is not? And why should its results be confined to those which are of immediate biological utility

to the species? Even micro-organisms could be said to enjoy the possession of a genetical system of high mutability—or anyhow of great evolutionary versatility—which will protect them just as effectively from antibiotics which have yet to be discovered as from those which they have coped with hitherto. And is not the evolution of a certain kind of nervous or antibody-forming system a safeguard against emergencies yet to happen as well as against those which prevail to-day? Amidst the minutiæ of specific *ad hoc* adaptations we must not lose sight of the evolution of genetic and physiological systems: natural selection is not such an improvident, hand-to-mouth affair.

One of the most popular misconceptions about evolution by natural selection is that which treats it as the *dénouement* of the following train of thought: (*a*) organisms produce offspring in numbers vastly in excess of their needs; (*b*) not all these offspring survive; therefore (*c*) only those survive which are the best equipped to do so, the "fittest". The catch in this plausible little syllogism (pointed out years ago by Fisher) lies in its major premise (*a*). So far from producing a vastly excessive number of offspring, most organisms produce the number approximately most apt to perpetuate their kind. Degree of fecundity is one of the consequences of natural selection: it is not its cause. Nidicolous birds, Lack tells us in his article, illustrate this truth with particular clarity, for they do in fact lay clutches of a certain size, although they could lay more eggs—the egg industry preys upon the inexhaustible gullibility of the domestic fowl—and could, of course, lay less. Having regard to all the exigencies of giving birth to and rearing eggs and young, the size of its clutch is just about that which gives each species its greatest likelihood of self-perpetuation. Lack's article in this book must stand as representative of the half-dozen odd that deal in part or in whole with birds, but it will not escape notice that the two other co-Fairy Godmothers of ornithology also appear in person. Mayr's article is of the distinction which, from him, we have come to take for granted; and it is most satisfactory, too, that Tinbergen should have made such convincing progress in the interpretation of a subject that was one of Huxley's earliest interests: the significance of courtship display in birds.

On the principle that those incompetent to criticise are impertinent to commend, I shall say nothing of the articles written by professional geneticists. It is more to the point to ask what geneticists themselves can learn from the remainder.

Hardy does well to remind us of the very profound contribution that Garstang made to our understanding of the modes of evolution: the idea that organisms may slough away the latter ends of their life-histories and build their lineages and adult lives anew upon larval or even embryonic forms. Indeed, I believe that neoteny, so far from being a curiosity, is an entirely fundamental tactic of the evolutionary process. "There is an impressive list", writes Hardy, "of animal groups which may have been evolved in this way: Siphonophora, Ctenophora, Cladocera, Copepoda, Insecta, the Chordata as a whole, and (within the Chordata) the Larvacea, Enteropneusta, Cephalochordata (*Amphioxus*), some lampreys, the recent Dipnoi, the Urodeles, the Monotremata, and Man himself." Molluscs are added, and I am surprised to see no mention of Nematodes and Rotifers, which have many of the stigmata of pædomorphic forms. These several

groups are of very unequal systematic standing, and very unequal too is the evidence which might entitle one to regard them as pædomorphic forms. Garstang's case was first built upon, and most convincingly argued for, the derivation of true chordates from animals akin to sea-squirts, and I believe that no well-informed zoologist now challenges the correctness of his view. I am therefore particularly sorry to see that Hardy should have made so much of Garstang's rash guess that *Amphioxus* (via an ammocoete larva) was a pædomorphic derivative of a lamprey-like or Cephalaspid form. The reasons given in favour of such a derivation are insubstantial; so gravely damaging an objection as the lack of neural-crest tissue and its derivatives in *Amphioxus* receives no mention at all. Needless to say, most of the affinities that were at one time considered good evidence of recapitulation can now be inverted and treated as evidence of pædomorphic transformation: Haeckel is still the hero, though his portrait now hangs upside down; but neither way up can the argument carry the slightest conviction unless it is backed by that thorough analysis of early embryonic development for which one looks in vain.

I fear also that I cannot share de Beer's enthusiasm for the evolutionary doctrines of Dr Jovan Hadzi, as they are expounded here. It is just possible to see the sense of a derivation of Ctenophores by neoteny from Polyclad Turbellaria; but the evolution of Anthozoa from Turbellaria, and of Turbellaria themselves from ciliate protozoans, indicates nothing more than that evolutionary speculators set themselves somewhat lower standards of scientific rigour than prevail elsewhere. There is indeed nothing that *proves* these derivations false; the trouble is that I can see not the faintest reason for supposing them to be true. According to de Beer, Hadzi makes a clean sweep of what might be called the "Coelenterate" theory.

> "Of all the views currently and generally held in Zoology, hallowed by a long period of acceptance and teaching it would be hard to find one more firmly established than that which holds that the most primitive Metazoa, the animals which evolved from the Protozoa and stand on the main line of evolution towards the higher Metazoa, are the Coelenterates."

I do not agree. Zoology would be in a sorry plight if the Coelenterate Theory of the origin of Metazoa were indeed one of its chief theoretical ornaments. The theory is not by any means a hallowed doctrine. It is a desperate guess made by those who have felt obliged to express some opinion on the matter. Others, more fortunate, remain silent, knowing that there is very little that can be usefully said on the matter at all.

It would be a salutary thing if no-one were allowed to indulge in phylogenetic speculation until a prolonged course of experimental studies had shown him how merciless facts can be to even the most plausible and passionately held hypothesis. Without that discipline, there is always a danger that the unravelling of phylogenies can degenerate into a kind of scholarly indoor pastime. If the energy at present dissipated in uncalled-for and unverifiable musings on the affinities of animals were directed towards seeking new methods of analysis, no-one would complain, however unsuccessful they might be; and although biochemical interpretations of

phylogeny have so far led almost nowhere, it would reveal poor judgment to suppose that a scrupulous analysis of protein and nucleoprotein structure and behaviour might not one day provide us with the clues we need.

It is pleasant therefore to turn to the present volume's principal scholarly document, Zuckerman on the interpretation of the descent of man, and in particular of the standing of the Australopithecines. Zuckerman's article will be read with pleasure by all those who, like myself, have contemplated with incredulous disgust the reasoning which purports, for example, to have revealed the intimate domestic habits of the Australopithecines. What Zuckerman attempts to do (admittedly on a tiny scale, compared with the grand affinities that have so far been in question) is to *reason out* judgments that have hitherto been entrusted to the act of intuition. The trouble with intuition, and with informed judgments based upon a lifetime's experience, is that these secret inner voices speak to their several owners in several different tongues ; so that, paradoxically enough, intuition reveals no truth more clearly than that the intuition of others is at fault.

The contributions that have been commented upon above must serve as a sample of the book's contents, but one more must be mentioned because it is of a very different kind : Young on memory, heredity and information. The terminology of information theory has caught on in many departments of biology, a good sign that it lives up to its subject matter by having something informative to say. Its principal virtue is that it has revealed an abstract general similarity of procedure in a wide variety of biological activities. The similarities, once they have been pointed out, are obvious ; once expounded, most good ideas are so. Thus to conceive of the hereditary process as a handing on of genetic information is, in my opinion, telling and to the point. Fastidious biologists do not speak of the " inheritance of characters " but may get involved in tedious circumlocutions in attempting always to speak of inherited character-differences. These terminological irritations can to some extent be mollified by speaking of the inheritance of genetic information ; and this has the added advantage that we may now easily conceive of the distinction between a secular change in the nature of the information that is transmitted and in the nature of the system of transmission itself. There are still some conceptual crudities in the biological applications of information theory. By what trick of topsy-turvy reasoning, for example, did it ever come to be suggested that the brain was a kind of (" endosomatic ") calculating machine, when the simple biological truth is that the calculating machine is a sort of exosomatic brain ? It performs brain-like functions, much as telescopes have eye-like and forceps hand-like functions, and motor cars the functions endosomatically performed by limbs. We *may* learn something about the brain by studying calculating machines, just as we have quite unmistakably learned something about the eye by studying glass lenses ; but it need not be so ; limbs do not work at all like internal combustion engines. Young and others are concerned to find out just how much can be learned of the working of the brain by using the concepts and practical findings of communications engineering. There is no *a priori* right or wrong about such an endeavour ; we can only judge it by how successfully, after trial, it proves to work.

P. B. MEDAWAR.

THE BIOCHEMISTRY OF GENETICS. By J. B. S. Haldane. George Allen and Unwin. 1954. Pp. 144. 15s.

The concepts of genetics and of evolutionary theory are of prime importance in the development of biochemistry. In its present state this subject is deeply concerned with the demonstration of impressive similarities in the enzymatic equipment of cells of living organisms of all classes, and it is as yet largely unable to deal with the variations in interaction of these items of equipment which must form the basis of the distinctions in form and function between different cells and species. Despite the sanguine pronouncements of some biochemists, reminiscent of the *fin de siècle* physicists, that all that remains is to tidy up, it is certain that more—and more difficult—biochemistry lies ahead than behind.

This book should be enough to convert any sceptic to such a view. Its author is uniquely qualified to discuss both biochemical and genetic aspects of the interaction between the disciplines, and he brings out very clearly the inadequacy of available biochemical knowledge to the explanation of the genetical complexities which he discusses. It is a pity that so much space has been given to human biochemical genetics, since the possibilities of fundamental analysis of either genetic or biochemical problems are so limited in this field, and since existing knowledge has recently been admirably summarised by Harris. There are occasional obscurities arising out of compression in other parts of the book. In view of the importance of the topic, the discussion on pp. 106-7 of the relation of nucleic acid to genes could profitably have been expanded. The author, as is his wont, treats the mathematical aspect of this problem too concisely for the general reader, and the important suggestion that only a very small fraction of the chromosome nucleic acid can be present in the genes themselves could well have been treated more fully.

R. A. Fisher has defined variance as the attitude of one statistician to another, and it seems probable that variation can be similarly defined in terms of geneticists. A biochemist may therefore be excused from judging the merits of the genetical aspect of this book, whilst being grateful for the introduction which it affords to the conceptual complexities of this subject in a context which is obviously his concern. If, as seems likely, the book also serves to introduce the geneticist to biochemical notions with which he will have increasingly to concern himself, then it should be very widely useful.

R. B. Fisher.

BLOOD GROUPS IN MAN. By R. R. Race and R. Sanger. Blackwell Scientific Publications. (August 1954, 2nd Edition.) 30s.

The second edition of this essential book has been awaited eagerly by all who are interested in serology, and no one will be disappointed in it. The authors are to be congratulated upon a remarkable achievement. They have brought their well-known text book up to date, when to do so required the inclusion of a large proportion of new data, and the consequent minor reorientation of many aspects of the subject. Their undoubted success is due partly to the fact that they are themselves in the front rank of research workers in this field, and that many of the discoveries which they review are their own. Consequently, this is an authoritative work, one which is indispensable alike to geneticists and medical men.

It seems almost ungrateful to criticise a book to which so many students will be indebted, but since further editions are certain to be required, it is possible that constructive suggestions may be of use.

The fundamental drawback to this subject is its defective notation, which makes it difficult to expound, to learn, and to analyse. This is not in accord in accepted genetic usage ; but, far more serious than that, it is inconsistent within itself, and is seriously misleading. The authors are not to be blamed for this, for they are merely adopting the conventions which are in general use. How serious is the situation may be judged from the fact that it is often impossible to determine, both in this and in other works on serology, whether a given symbol refers to a gene or an antigen. A few instances only can be mentioned here to indicate the confusion that exists. Thus the genes of the Duffy and Kell systems are represented respectively as Fy^a, Fy^b, and as K, k. Though the notation used is so dissimilar, the situations involved are the same in that each allelomorph produces an antigen recognisable both in single and in double dose. On the other hand, P and p are used for the genes of the P series, being the same type of notation as K, k, though the serological conditions are different ; for the antigen controlled by p (if it exists) has not yet been detected. One further example out of many must be mentioned : the extraordinary fact that the genes of the MN groups are themselves represented as M and N: an arrangement which gives no indication of their allelomorphism, and so breaks the most fundamental rule of genetic notation.

Turning now to other subjects, the diagrams of crossing-over on page 169 are misleading because chiasma formation is incorrectly represented as occurring in the two strand, instead of in the four strand, stage. On seeing them, those unacquainted with the details of meiosis, and it is surely for such that these figures have been prepared, are bound to conclude that if a single cross-over were to occur between two loci in a hundred per cent. of instances, one hundred per cent. of inter-change chromatids would be formed, instead of the correct value of fifty per cent. This could lead to serious errors in the analysis of linkage.

There are a certain number of subjects which merit rather more extended treatment here than they receive. For instance, those interested in the more recent developments of serology will turn, among others, to the section describing the F locus of the Rhesus system, its genetics and the antigens and antibodies which it controls. Yet the information to be obtained under the heading *The inheritance of f* (p. 160) is confined to the statement, " The inheritance of *f* is that to be expected of an *Rh* antigen. An example will be given in the next section ". This example proves to be a small family tree in which segregation at the *f* locus is taking place. It throws no light on its mode of inheritance, and certainly does not indicate the unexpected fact that, contrary to accepted genetic usage, the notation has so been chosen that *f* is dominant to *F*.

Finally, I would strongly recommend that the references be listed alphabetically under authors, instead of appearing in the order in which they happen to be quoted in the book. It troubles no one that the first reference one meets in reading the text is not number one, but the corpus of an author's relevant works is a valuable component of a book such as this. Here, the contributions of each author must be searched for among twenty-one distinct bibliographies (at the end of each chapter), a system

which some defend, but I deplore ; but even within each bibliography they appear scattered at random, sometimes among a hundred entries or more, an arrangement which is not easily defensible.

These, however, are minor criticisms. It would be unnecessary to make them, were not the quality of this book a matter of consequence to a wide circle of biologists and medical practitioners. E. B. FORD.

THE DISTRIBUTION OF THE HUMAN BLOOD GROUPS. By A. E. Mourant, M.A., D.Phil., D.M. With a foreword by H. J. Fleure, F.R.S. Oxford : Blackwell Scientific Publications. 1954. Pp. xvii+438, 4 text figures, 9 maps. 42s.

In the second edition of *Genetics and the Origin of Species*, published in 1941, Dobzhansky states : " The geographical distribution of the blood-groups in man has been studied in more detail than any other instance of geographical variability ". Dobzhansky, like many other workers since that time, was able to consult Boyd's invaluable compilation, published as a volume of Tabulæ Biologicæ in 1939, which covered all anthropological blood-group data up to 1938. At that time only three systems were known : ABO, MN and P. Data on MN were somewhat scanty and the P system was, and remains, difficult to work with. During the past sixteen years the accumulation of further data has become enormous. Published ABO groupings run into millions ; six new blood-group systems have been discovered, all of which show large or very large differences of gene frequencies in different parts of the world ; the Rhesus system, with its genetic unit of three (now four) closely linked loci, provides a wide variety of genetic combinations, as also does the now extended MNSs system. Dobzhansky's remark could indeed be repeated to-day with even greater emphasis. The urgency of bringing Boyd's tables up to date has long been apparent, though of course the immense amount of work that would be involved was equally apparent. Boyd had indicated that he had no immediate intention of doing so himself and the task has now been accomplished in Mourant's anxiously awaited monograph. It is hardly necessary to mention Mourant's qualifications for the work. He is himself the discoverer or co-discoverer of two of the blood-group systems, to say nothing of a host of other contributions. His knowledge of every aspect of the serological field is encyclopædic, and he has long been specially interested in anthropological applications, involving detailed knowledge of a very large and scattered literature.

The book, then, must first be judged as a work of reference. We are told that it was originally intended to publish complete tables for the ABO system (after 1938). This proved too great an undertaking. Instead there is the next best thing—a complete bibliography, very conveniently arranged, with titles of papers quoted, and with its own topographical index. It is to be hoped, however, that it will ultimately prove possible to produce a supplementary volume of ABO tables, which would be of great value to many workers. For the MN system after 1938 and for all the other systems complete tables are published. These cover 80 pages. Much thought has been given to form and content and the result is excellent. The basic plan is to give the total number in each sample, with observed and expected percentage phenotypic frequencies and calculated gene frequencies. It must have been a difficult choice whether to show

group numbers or percentages ; to have shown both would have taken up too much space. The plan adopted has the advantage that the reader can compare percentage frequencies from different areas directly, while for those who want to work on the data it is a simple matter to reconstruct the original figures. The data for Rhesus are clearly grouped according to the number and kinds of antisera used, so that no ambiguity seems possible. Altogether it seems probable that the standards of completeness and accuracy, both of the bibliography and of the tables, are as high as is ever likely to be attained in an undertaking of this kind. A special word of praise should be added for the attractiveness of the lay-out and the resisting of any temptation to sacrifice clarity for the sake of saving space.

Rather more than half the book consists of a review of the present state of knowledge. The genetics of the blood-groups are briefly reviewed ; for a fuller account the reader is referred to Race and Sanger's companion volume *Blood Groups in Man*, now in its second edition. The leading variations in frequencies of the various systems throughout the world are clearly summarised without being obscured by too much detail. There is a number of excellent maps. Certain other simply inherited, or fairly simply inherited, traits are wisely included, for example, sickle-cell anæmia, Mediterranean anæmia, the ability to taste phenylthiocarbamide and the ability to smell hydrocyanic acid. It is a pity, incidentally, that this last Mendelian difference, which is sex-linked, should involve work with so dangerous a compound. There are also chapters on the grouping of bone and tissue specimens ; on the blood-groups of animals ; on the collection, preservation and transport of samples ; on the simpler methods for calculating gene frequencies ; and, finally, on some recent discoveries made after the rest of the book was written.

In *Penguin Island* Anatole France makes a remark to the effect that history is a simple matter as long as there is only one historian. With the blood-groups anthropological deductions were certainly easier as long as knowledge was effectively confined to one system, namely ABO. Not of course that it could be expected that all the blood-group systems would tell the same story ; with the exception of Lewis and Lutheran, which are genetically linked, different chromosomes are involved. There is doubtless a complex pattern of balanced polymorphism and indeed evidence is now beginning to appear showing quite high selective values, apart from antigenic properties. One or more systems may tell one story, another system a second story, others may give parts of a story. The B gene indicates outward spread from central Asia and perhaps also from Africa ; the O and A genes the successive waves of invaders in the British Isles ; one Rhesus compound gene, cDe, very common in Africa and nowhere else, gives a clear picture of negro influence ; many systems combine to indicate the uniqueness of the Lapps and the Basques. Clearly attempts at synthesis are very difficult at present. Mourant makes that attempt in one chapter, but he can hardly be blamed if the result is somewhat nebulous. More data yet will have to be collected, and gene frequency changes in response to selective pressures and environmental differences better understood, before a convincing synthesis is likely to be forthcoming. Nevertheless, even if emphasis has shifted away from genetic drift and towards selection there are plenty of indications that changes in gene frequency must be slow. As Mourant points out, the Basques and the Lapps, known to be distinct

ethnic groups, are highly individual in many of their frequency patterns, while by contrast Western Europe as a whole, with all its varied environmental conditions, presents several pictures of broad uniformity. What evidence there is points to very low rates of mutation of blood-group genes, and very large bodies of ABO data do not reveal any difference of frequencies over an age span of two generations or more. There is, in fact, every reason for continuing to believe that blood-group frequencies can provide valuable evidence on human ancestry and migration over a period of at least some thousands of years.

The Rhesus maps are perhaps open to criticism. These show the frequencies of the elementary genes, C, D and E, which are serological and not genetic units. Maps showing frequencies of the compound genes would be more meaningful, though it is easy to see the difficulty of providing them when the data refer to various levels of completeness, ranging from the use of one antiserum upwards. It is to be hoped, however, that they can be included in the next edition.

The book can be warmly recommended to anthropologists and geneticists as well as to the general reader who wants to have a clear, up-to-date account of the anthropological aspects of the blood-groups. To the research worker the tables, the bibliography and the practical directions will prove invaluable.

J. A. FRASER ROBERTS.

HUMAN HEREDITY. By J. V. Neel and W. J. Schull. The University of Chicago Press. 1954. Pp. 361. $6.

The great advances that have been made in our understanding of the genetics of the human blood groups during the past decade and the changing emphasis in medical research from infectious and contagious disease to pathological conditions in which genetic factors are of more importance have led to a considerable expansion of interest and work in human heredity. In North America, in particular, a number of centres for research, teaching and counselling in human genetics have been set up in recent years and, of these, one of the largest is the Heredity Clinic at the University of Michigan. Drs Neel and Schull, two members of this clinic, have now written a book on human heredity for the "advanced and competent student in biology and medicine and for researchers and theoreticians in this field." There are few texts on this subject and one that contributes to an understanding of the principles and methodology must certainly be welcome.

The book commences with eight chapters devoted to the bases of genetics and probability theory. These are followed by nine chapters dealing with the genetic theory of continuous variation, the detection of linkage in man, mutation, physiological genetics, statistical estimation and tests of significance, analysis of family data, population genetics, twins, genetics and epidemiology (in this order), and finally three chapters on genetic counselling, eugenics and medico-legal applications. The reader will perhaps be surprised that there is no chapter devoted to blood groups but, if he is patient, he will find much of the relevant material scattered in chapters 8, 13, 15 and 19.

From the start the authors recognise that a knowledge of both genetics and statistics is essential to the study of human heredity. The elements of genetics are introduced using human material but the account is not complete and the reader should have some prior knowledge of genetics. He should also be familiar with the calculus and the elements of biometry.

The authors are at their best when writing about their clinical experience in human heredity. A score of new pedigrees and several case histories from their clinic are presented and these are full of interest. In the more theoretical chapters, the authors express themselves with much less conviction.

On page 245, we read, in connection with the Rhesus blood groups, "The apparent magnitude of the selection involved is . . . very considerable, suggesting an unstable system". This is very misleading. Quite apart from the Rhesus situation, the presence of large selective forces suggests very definitely the existence of a stable balanced polymorphism. But no more than ten lines are given to this important concept of a selectively balanced polymorphism, which is dismissed as "a hypothesis difficult to prove for man".

In the chapter on linkage, it is erroneously stated that the maximum likelihood scoring method does not lead to efficient estimates of the recombination value. On the strength of this assertion, the authors dismiss the estimation problem and confine their attention to the sib-pair method of linkage detection. This is truly an inefficient method of detection and one that should rarely be used and not at all for "characteristics having a somewhat obscure but nonetheless real mode of inheritance" (p. 123). The added disadvantage of the sib-pair method that data from heterogeneous populations cannot be combined and analysed is not mentioned.

It should be noted that an unmentioned assumption in the model developed in chapter 9 to elucidate the genetic basis of continuous variation is that random mating is taking place within the F_1 of two homozygous stocks. Otherwise, the statement (p. 101) "If the initial gene pool is very large, then we may assume that p (gene frequency) is constant for all loci" is not justified.

Misleading statements are to be found in a number of other places. Genes themselves are said to be homozygous or heterozygous. Estimates are said to be formed of genes instead of gene frequencies. Maximum likelihood estimates are said to be consistent and efficient "almost always" instead of always. On page 221, a distinction that is not a distinction is described as a logical one.

The "advanced and competent student" to whom this book is directed possibly will not be troubled by such things. But what of the less experienced readers whom the authors presumably have in mind on page 22 where they set out "to dispel a common misconception that what is inherited is fixed and immutable"?

The great amount of material which has been brought together in this book should have been sifted, sorted and presented more carefully. As it is, the text is an important addition to the small general literature on human heredity but it needs to be read critically. J. H. BENNETT.

THE GENETICS OF *PARAMECIUM AURELIA*. By G. H. Beale. Cambridge University Press. 1954. Pp. 176. 12s. 6d.

The existence of genetic restrictions to mating in *Paramecium aurelia* was discovered by Sonneborn in 1936. Since that time he and his pupils have carried out a study of this group of organisms so fruitful as to react upon the whole development of biology. Now Dr G. H. Beale, one of Professor Sonneborn's former colleagues, gives us an account of the results.

The genetic study of *Paramecium* breaks new ground in two ways. It introduces old and established genetic principles on the new level of a unicellular organism. And it reveals on this new level new principles fundamental to the relations of genetics and physiology. This makes the task of presenting *Paramecium* genetics to the general biological reader almost as difficult and almost as important as the task of discovering it. At the beginning of such a presentation the writer has to decide what are the correct analogies between the very complex genetic system of the unicellular infusorian and those somewhat simpler or somewhat better understood systems that are known in the fungi and in the higher plants. He has to make this decision at the beginning because it governs the terminology that he uses for the systematics of the organism and for the elements of its genetic system with all their implications.

What are the terms used in *Paramecium*? In *P. aurelia* there are eight inter-sterile groups. In the past they have been described by *Paramecium* workers as *varieties*, but they are now known to be good species merely waiting to be described. Dr Beale gives the facts but does not apply them.

Within seven of these *sub-aurelian* species all individuals fall into pairs of alternative and parallel groups of a plus-and-minus type between which there is mating and within which there is no mating. What group a line or clone falls into depends on the properties of its macronucleus, sometimes in reaction with its cytoplasm. Now this system in its causation must be peculiar to organisms with a macronucleus. But in its evolutionary effects and perhaps in its physiological mechanism it is analogous with the incompatibility systems of higher plants and with the heterothallism of fungi. Like the second of these it has, on a false analogy, been described as " sex ".

These groups are not described by Beale as incompatible groups but as " mating types ". Why? Because, it is said, *Paramecium* undergoes a process of autogamy which is equivalent to self-fertilisation and we cannot have self-fertilisation and cross-incompatibility in the same individual. Now in its effects autogamy is certainly related to self-fertilisation. But not in its inherent character. It involves no conjugation of cells. It involves a union of identical haploid nuclei within one cell. It is analogous to the fusion of such nuclei in the parthenogenesis of certain higher plants and animals. Why then should we be surprised to find that it is consistent with self- and cross-incompatibility?

Symbols, by the way, are also important. The system adopted, or rather, retained, by Beale for these various genetic groups, however, is difficult for the beginner. And it is unnecessarily so. First, the " varieties " (or species) are labelled with arabic numerals. Secondly, the incompatibility groups (or mating types) are given roman numerals with a sequence of their own independent of the " varieties " and disregarding Sonneborn's plus-and-minus simplification. Thirdly, the stocks (which are not named under their " varieties ") are given a mixture of arabic

numerals and capital letters. This confusing system arises from the gradual history of *Paramecium* genetics. But why not use the obvious and agreed simplifications?

There is another analogy between *Paramecium* and the higher organisms which does not concern terms or symbols but does concern the place of *Paramecium* in the methodology of genetics and also in our understanding of genetic systems in general. In *P. aurelia* there is, as we have seen, a combination of diploid incompatibility with haploid selfing. This means in effect that there is an alternation—a facultative alternation—of sexual generations of two kinds: one reproduces by autogamy and consists therefore of constant and absolutely homozygous diploid individuals, and the other reproduces by enforced cross-breeding. Such an alternation of self-fertilisation, which by a single stroke produces the purest of pure lines and cross-fertilisation producing heterozygosity, exactly answers to the formula discovered by Mendel for the crucial analytical experiment in genetics. Thus *Paramecium* by its nature and of its own accord has the capacity of carrying out with extreme precision the very experiment which the geneticist needs for defining its nuclear or Mendelian heredity. It is in part to this capacity for *in-and-out*-breeding that we owe the rigour with which it has been possible for the *Paramecium* school to separate the nuclear and cytoplasmic components of heredity.

Dr Beale describes the three main fields of cytoplasmic heredity in *Paramecium*: kappa, the antigens, and incompatibility. He is interested in showing that the kappa particles are unique. They propagate themselves in their hosts like viruses but they rarely or perhaps never exchange hosts by natural infection. They may therefore never have exchanged hosts in the past. Moreover, the substance paramecin which they produce, although it contains nucleoprotein, does not, as a virus would, propagate itself but disappears in its victims. Thus the kappa particles are not viruses. And the evidence is against supposing that they ever have been viruses.

In spite of their uniqueness, however, the kappa particles help to show us how the more easily classifiable particles in the cell live and move and have their being. Particularly they broaden our understanding of what the reviewer has called the *corpuscular* elements of cytoplasmic heredity. They make it clear that the genetic variations in size, stability, efficiency, temperature tolerance, rate of propagation and nuclear subordination shown by the plastids in plants can be shown by a corpuscle having quite different functions in the cell. These questions have been discussed by the *Paramecium* workers and Dr Beale might well have mentioned them.

The external reactions of the cytoplasmic systems responsible for the antigens and for incompatibility in *P. aurelia* are shown in regard to temperature, salinity, homologous antisera, enzymes and irradiation. Beale points out how such a range of responses in free unicellular organisms enables us to picture the process of differentiation in higher organisms, and the changing competence of their cells to respond to the external stimuli of organised development. But the importance of this example is surely enhanced when we find that there are alternative courses in the development of higher plants and that external conditions, especially temperature, will sometimes switch the balance of cytoplasmic determinants from one to the other. In *Tradescantia* (La Cour, *Heredity*, *3*, 319) the difference between large and small pollen grains seems to be of this type.

And in the tomato, potato and pea (*cf.* Lewis, *Heredity*, 7, 337) the difference arises between type and rogue. Equally in *Paramecium* and in these plants we are dealing with alternative steady states and we are also dealing with what we still have to describe as the third level of heredity, the " undefined residue of heredity ", undefined because it is non-corpuscular and susceptible to change during differentiation. This would also have been worth mentioning.

Many of the analogies between *Paramecium* and the higher organisms merely suggest new lines of enquiry. The mutual exclusiveness of cytoplasmic states in *P. aurelia* recalls the property of suppressivity shown in crosses between green and pale *Scolopendrium*. The breakdown of a cytoplasmic state when meiosis and nuclear fusion occur recalls the frequent failure of viruses to survive the sexual process. On the other hand the parallel antigenic polymorphism in the different " varieties " and the special functions of the macronucleus, these are properties so far peculiar to *Paramecium*.

Since this book is bound to interest many who are not generally concerned with genetics Dr Beale is wise to offer us some introductory remarks on the chromosome theory of heredity. He is not perhaps so wise in the choice of what he tells us. On page 1 he suggests that Mendel " ignored " the transmission of material in heredity. But on page 5 he quotes Mendel's own reference to the elements in the cells responsible for heredity. On page 1, again, Dr Beale argues that the hereditary materials in one organism " can under no circumstances embody the same atoms and molecules as were contained in the parents ". This is a surprising statement. But supposing that we accept it what has it to do with *Paramecium*? On page 2 we learn that an " individual scarcely inherits any material possessions at all but merely some abstract pattern ". Presumably *Paramecium* is a concrete pattern, its chromosomes and its kappa particles are an abstract pattern. On page 3 Beale continues with the idea that in the past it has proved " essential " to separate the hereditary material from the rest. Nevertheless it may be " unreal " to do so and it may not be essential in the future. On page 4 Beale reveals another difficulty: Weismann had " no real *proof* (sic !) that the chromosomes had any connexion with hereditary processes ".

These remarks are a not very satisfactory recapitulation of the views expressed by Bateson forty-five years ago, views which so soon became untenable. Yet they are used to introduce *Paramecium* ! They are on the first pages of a book in which Dr Beale himself interprets this organism on the assumption of an unimpeachable nuclear continuity, an organism in which spontaneous gene mutation has never been demonstrated, an organism therefore enjoying a molecular stability in the gene greater than that to be expected in a bar of iron.

Dr Beale makes another stumbling block for his readers when he suggests that it is only " hereditary characters studied *by the Mendelian methods* " which are known to be determined by genes (p. 7). We know of studies of the relation of balance, polyploidy, segregation in hybrids, fertility, induced mutation, continuous variation and polygenic inheritance in which the assumptions of breeding experiment and chromosome study have been combined. The methods used are not Mendelian. But do they not provide a large part of the strength of the chromosome theory and a larger part of the evidence of what the chromosomes and genes are

doing in heredity and evolution? And have they not in fact contributed to the understanding of *Paramecium*? Surely they have explained to us the varied properties of the polyploid macronucleus and the special consequences of irradiation.

These doubts and misgivings on fundamental questions have somewhat distorted the evidence and obscured the argument of this book. Dr Beale has tried to fit the revolutionary discoveries of the American geneticists into a framework for which they themselves have no use. His purpose is genuine, his methods are rigorous and often most fruitful, but his outlook seems to have required him to throw away some of the best of what his efforts have won.

C. D. DARLINGTON.

GENETICS BIOLOGICAL INDIVIDUALITY AND CANCER. By C. C. Little. London: Geoffrey Cumberlege, Stanford University Press. 1954. Pp. 111. 20s.

This book traverses the field of cancer research with a series of somewhat disconnected but often remarkable statements. The following example is worth quoting (p. 89) :—

" When a break in linkage occurs it is called *crossing over* because during mitosis a gene exchanges place with its counterpart gene in the other member of the chromosome pair to which it belongs."

C. D. D.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND SEVENTEENTH MEETING of the Society, held on TUESDAY, 29th MARCH 1955, at WYE COLLEGE, Near Ashford, Kent

THE ARTERIAL SYSTEM OF THREE INBRED STRAINS OF MICE

M. D. FROUD
Wye College, Kent

The arterial system has been studied in 25 specimens of each of three inbred strains of mice (C57BL/Gr : CBA/Gr : A/Gr) by means of rubber latex injection and subsequent clearing in benzyl alcohol. There are at least 18 well-defined differences between strains in the arrangement of the arteries and these occur in all parts of the body. In some cases the alternative arterial patterns of a particular region are confined to one strain : in other cases there is a slight overlap and the pattern normally found in one strain occurs occasionally in the other two. Similar variations in the arterial system have been described in other species of mammal and in human anatomy and attributed to unknown factors in development. It is clear that, at least in the mouse, they are to a varying extent under genetic control.

ANALYSIS OF HETEROKARYOSIS IN A WILD HOMOTHALLIC ASCOMYCETE

H. SUBAK SHARPE
Department of Genetics, Glasgow University

Heterokaryosis has not been previously reported in a wild homothallic ascomycete. Only two out of fifteen *Aspergillus glaucus* isolates from the wild proved to be heterokaryotic. It is suggested that the low incidence of heterokaryosis in nature in a sexual homothallic ascomycete compared with the high frequency reported in asexual fungi by other workers is no accident.

Growth rate of colonies from conidia (asexual) and from ascospores (sexual), the latter both from selfed and crossed perithecia of the heterokaryons, was measured on two different media. In both isolates naturally occurring single gene differences served as markers and total growth in the time interval from 48-192 hours was analysed. Different situations were found in the two isolates. Isolate 29 is a stable heterokaryon whose two constituent nuclear components differ in a single gene or supergene. Line 26 is a very unstable heterokaryon on all media tried whose two constituent nuclear types differ in many genes. The distributions of growth rates of colonies from ascospores having crossed origin indicate control by several polymeric genes or polygenic blocks. These colonies show continuous variation also in morphological characters.

Thus in nature heterokaryons occur with component nuclei differing by several genes.

SPONTANEOUS NATURE OF A HERITABLE CHANGE TO PRODUCTION OF PENICILLINASE

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The study of the production of enzymes offers many advantages for understanding the specificity of both proteins and genes. Bacteria are ideal for enzyme studies and although less suitable for genetical analysis, one may obtain results capable of a straightforward interpretation by appropriate techniques.

An inherited change from penicillinase sensitivity (due to the inability to produce penicillinase) to penicillin resistance (due to production of large amounts of this enzyme) has been found in *Bacillus cereus*. This change can be proved, by the velvet pad replicate technique, to be a spontaneous event, not caused by the penicillin treatment. Using this method penicillin-resistant variants can be obtained, and the penicillinase production proved, without ever bringing the cells into contact with the antibiotic.

ON THE TECHNIQUE OF MUTATION EXPERIMENTS WITH *DROSOPHILA*, ESPECIALLY BY THE FEEDING METHOD

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Institute of Animal Genetics, Edinburgh

Factors which affect, often drastically, the result of mutation tests with formaldehyde-food are : nutritional value of the food ; the presence of harmful substances in the food ; storing of the treated food before use ; degree of crowding of the larvæ. All these appear to act indirectly through their effect on developmental rate, which in its turn determines the degree and distribution of sensitivity in the larval testis. Differences between strains are marked, but their origin has not yet been analysed. After about 24 hours on formaldehyde-food, larvæ cease to respond to both its toxic and mutagenic action ; this is partly due to deterioration of the food and partly to " adaptation " of the treated cells. Heterogeneity of response between treated larvæ is negligible with formaldehyde, but may be the main reason for the low effectiveness of pyronin as a mutagen. A small increase of mutation rate after addition of a chemical to the food may be due to some general effect on metabolism rather than to a specific action of the substance used ; it is sometimes possible to distinguish experimentally between these alternatives. The sensitivity pattern of the testis to different mutagens is not the same ; it may vary even for mutagens given in the food. This has to be taken into account in quantitative comparisons between mutagens. Still further, uncontrolled conditions may cause fluctuations in mutation rate, so that the real accuracy of estimates of spontaneous or induced mutation rates is less than the statistical error in a single test would suggest. A number of medium-sized tests, with controls, are therefore much preferable to one or two large-scale ones for establishing whether a controlled variable, such as type of treatment or age at treatment, influences mutation rate.

ABORTIVE TRANSDUCTION OF MOTILITY

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When motility was *transduced* from wild-type (motile) *Salmonella* to non-motile (non-flagellated) strains appearances suggested that a phage-imported genetic factor for flagellation sometimes displaced its " allele " determining non-motility in the recipient cell, which would consequently produce only motile progeny, and sometimes failed to displace its " allele ", instead persisting as a non-replicating,

" supra-numerary gene " in (one of) the descendants of the recipient cell ; if the factor is dominant to its " allele ", that descendant containing it would be motile, the others non-motile.

Individual cells made motile by transduction and isolated by micromanipulation give pedigrees generally consistent with these hypotheses ; but it seems that when the cell containing the " supra-numerary gene " divides, the daughter which fails to obtain the " gene " is yet motile, because it receives a few " motility-conferring particles " (probably flagella, or their basal granules), which themselves persist, but do not multiply, within the clone this daughter cell produces. A cell containing *one* such particle is weakly motile, and no more than *one* of its descendants is motile. A cell containing the " gene " is more motile, and produces a clone containing many motile bacteria, *e.g.* c.40 in a clone of c.10^4. A non-replicating " gene " has been traced for c.22 generations ; a " particle " for c.27 generations.

SEGREGATION WITHIN INBRED LINES OF CHICKEN

A. G. COCK
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By examining F_1 crosses in this country and in U.S.A., hypostatic colour factors carried by inbred lines of two white breeds have been identified. In White Plymouth Rocks (recessive white) 11 out of 16 lines are segregating at the **E**-locus and 6 out of 16 at the **S**-locus (sex-linked). In White Leghorns (dominant white) 6 out of 12 lines are segregating at the **E**-locus and 3 out of 7 at the **B1**-locus. The coefficients of inbreeding of the lines vary from 37·5 to 98·7%. The amount of segregation occurring is greater than could reasonably be expected in the absence of selection favouring heterozygotes. A comparison is made with similar results relating to blood-group loci obtained by other workers.

EXTREME ASYMMETRY IN RESPONSE TO SELECTION

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Extreme asymmetry of response has been encountered in the two-way selection of mice for lactational performance. The realised heritability for continued upward selection is almost zero, while for back selection it approaches unity. In the low line, however, the responses to continued and to back selections are equal and intermediate. Daughter-dam regressions in the two lines are equal.

Models are discussed which may throw some light on the causes of this asymmetry.

DISTURBED SEGREGATIONS OF AN ALBINO IN LUCERNE

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Lucerne (*Medicago sativa*) is a tetraploid ($2n = 4x = 32$) and data published by Stanford of experiments designed to test whether segregation is disomic or tetrasomic have clearly shown that it is tetrasomic. It has, however, also been suggested that other loci might show disomic segregation.

In the present case segregation appears to be tetrasomic, with the ratios disturbed by two contrary influences. The albino embryos frequently fail to produce mature seeds and there is an overall deficiency of albinos. But a duplex plant selfed gave a significant excess of simplex offspring ; taken with other evidence this suggests that the albino allele interferes with self-incompatibility.

The implications of this for lucerne population structure and for self-incompatibility are discussed.